
INVESTIGATION OF MICROORGANISMS IN INFECTED DENTAL ROOT CANALS

E. Ercan¹, M. Dalli², İ. Yavuz³, T. Özekinci⁴

Kırıkkale University, School of Dentistry, Department of Operative Dentistry, Kırıkkale, Turkey¹

Dicle University, School of Dentistry, Department of Conservative Dentistry and Endodontics, Diyarbakir, Turkey²

Dicle University, Faculty of Dentistry, Department of Pediatrics, Diyarbakir, Turkey³

Dicle University, School of Medical Faculty, Department of Microbiology, Diyarbakir, Turkey⁴

ABSTRACT

Objective: The purpose of this study is to investigate the type of microorganisms isolated from necrotic pulp tissues and from failed endodontic treatments in infected root canals. Methods: This study is based on an experiment conducted on 90 patients between November, 2002 and November, 2003 using a sampling. One hundred single root canals were microbiologically sampled from these patients by using sterile paper points. Among 100 canals sampled, 61 had primary infection and 39 had a history of secondary infection. Microorganisms were isolated and identified by using established advanced microbiologic techniques for anaerobic species. Results: A total of 197 cultivable isolates were recovered, the microbial findings in both types of canals, such as the number of species isolated, the 68.0% of Gram-positives and 27.9% gram-negatives, the 52.8% of facultative and 43.1 % strict anaerobic microorganisms 4.1% fungi and so on. Ten canals presented no microbial growth. Conclusions: The prevalence of bacteria and fungi found in 100 root canals is the prevalence of the microbial genera in primary and secondary endodontic infection. Peptostreptococcus spp was the most predominantly isolated microbial genera, followed by Streptococcus spp (14.2%), Porphyromonas spp (12.2%), E. faecalis (9.6%), Staphylococcus salivarius (8.6%), Prevotella spp (8.1%), Lactobacillus spp (7.1%), Actinomyces spp (7.1%), Candida albicans (4.1%), Fusobacterium spp (3.6%) Veillonella spp (2.5%), Eubacterium spp (2.5%), Bacillus spp (2.0%), and Escherichia coli (1.6%) were other types of bacteria recovered.

Introduction

The role of bacteria in the initiation of pulpal and periapical inflammation has been well demonstrated (12, 7). The success of endodontic treatment is directly influenced by elimination of microorganisms in infected root canals. It is well known that microorganism colonizing in an oral environment can be conducive to pulpal and periapical pathosis (2).

A number of studies have reported on the microbial composition of necrotic dental pulps. As such, in an infected root canal;

one or more bacterial pathogens could be isolated (6). Early studies has indicated primarily shown that the presence of facultative anaerobic bacteria are usually present in an oral environment. These otherwise strong studies, however, have failed to isolate obligate anaerobic microorganisms, probably due to the lack of an appropriate protocol to handle and cultivate difficult anaerobic flora at that time (1, 3).

Primary infected root canals are untreated canals where microorganisms are able to access and colonize the pulpal tis-

sue and impair its function (26). Secondary infected root canals indicate a failure of the endodontic treatment, especially due to the persistence of microbial infection in the root canal system (16, 18).

Several studies have shown that obligate anaerobes predominated in infected root canals and made up as much as 90% or more of the microbiota (9, 29). Dental canals with necrotic pulp tissues (primary infected canals) present a polymicrobial flora characterized by a wide variety of combinations of bacteria, averaging 4-7 species per canal. These bacteria are predominantly anaerobic and gram-negative (6, 23, 24, 25, 17, 5). On the other hand, recent works investigating the microbial findings of teeth with failed endodontic treatment have reported a very limited assortment of microorganisms, with predominantly facultative anaerobic gram-positive species, especially *Enterococcus faecalis* (6, 28, 15, 8, 14, 13).

The purpose of this study was to investigate the presence of bacteria and fungi in 100 canals using culture technique from primary and secondary necrotic (i.e. retreatment cases) pulp tissues and to identify such microorganisms.

Materials and Methods

Patients Selection

A prospective study was conducted at the Endodontic Clinic at Dicle University, Turkey, for evaluating microbial agents in infected teeth. This study is based on an experiment conducted on 90 patients between November, 2002 and November, 2003 using a random sampling. One hundred single root canals were microbiologically sampled from these patients by using sterile paper points. Among 100 canals sampled, 61 had necrotic pulp tissues (primary infection), and 39 had a history of failed endodontic treatment (secondary infection). Microbial samples were isolated and identified by using established advanced microbiologic techniques for an-

aerobic species. Patients who had received antibiotic treatment during the last three months or had a general disease were excluded from the study. Patients younger than 17 years old and older than 55 years old were also included. A detailed medical and dental history was obtained from each patient.

Clinical and sampling procedures

Samples were collected using strict asepsis proposed by Möller (10). The teeth involved were individually isolated from the oral cavity with a previously disinfected rubber dam. A two-stage access cavity preparation was performed by employing sterile burs and manual irrigation with sterile saline solution was used instead of water spray. All coronal restorations, posts and carious lesions were completely removed. The tooth and the surrounding field were cleansed with 30% hydrogen peroxide and then with a 2.5% sodium hypochlorite solution for 30 s. The solution was inactivated with sterile 5% sodium thiosulfate. Aseptic techniques were used for instrumentation during access to the root canal and a sample was taken from the same canal. The root filling was removed using Gates-Glidden drills (Maillefer Instruments, Ballaigues, Switzerland) and endodontic files without the use of chemical solvents. Irrigation with sterile saline solution was performed in order to remove any remaining materials and to moisten the canal prior to sample collection. The canal length was measured with an electronic apex locator (Root ZX, J. Morita Corporation, Osaka, Japan) with a small sterile file. The canal was instrumented within 0.5-1.0 mm of electronically determined apex, with a 20 K-file, whenever possible, and without the use of any irrigant. A sterile paper point was then placed in the full length of the canal and retained in that position for 60 seconds for microbial sampling. The canal orifice was flushed with nitrogen gas during the sampling process.

After the sampling period, the paper

point was immediately placed in a transport medium containing 3 ml of sterile reduced transport fluid and then submitted to the microbiology laboratory within 15 minutes. The maximum time between sample collection and laboratory processing was 4 h.

Microbial isolation and species determination

Inside the anaerobic workstation, the tubes containing the transport medium were shaken in a mixer for 60 seconds. Serial 10-fold dilutions were made up to 1/104 in pre-reduced Fastidious Anaerobe Broth (FA B; Lab M) and 50 μ l of each serial dilution were plated onto several media, as follows: 5% defibrinated sheep blood-FAA alone, and supplemented with nalidixic acid (0.001%, w/v), with nalidixic acid and vancomycin (0.00025%, w/v), and with neomycin (0.0075%, w/v) for anaerobes; 5% defibrinated sheep blood Columbia agar (OXOID, Hampshire, UK) plates for aerobes; and Sabouraud agar (OXOID) supplemented with 100 μ g/ml of chloramphenicol for yeasts. For anaerobic culture, the plates were incubated at 37°C in an atmosphere of 10% H₂, 10% CO₂ and 80% N₂ for 2, 5 and 14 days. Columbia agar plates were incubated aerobically at 37°C for 2 days, and Sabouraud agar plates were kept at room temperature for up to 5 days.

After incubation, each plate was examined and the different colony types subcultured non-selectively onto blood-FAA plates to obtain pure culture. The colonies were selected for further study based on appearance. The pure cultures were then Gram-stained, tested for catalase production and their gaseous requirements established by incubation for 2 days aerobically and anaerobically.

Bacterial colonies were subcultured for purification and identification. Anaerobic strains were identified by phase-contrast microscopy, morphotyping, Gram staining characteristic, and biochemical tests using enzyme-based kits (Rapid ID 32 Strep, API Staph, API C Aux (BioMérieux SA,

Marcy l'Étoile, France). Facultative anaerobic and aerobic organisms were identified by Gram staining, micromorphology and colony morphology, growth on selective media and biochemical tests using specific sets of enzyme-based systems (RapID STR and ANA II systems, IDS; API Strep System, bio Mérieux) where appropriate. Additional tests were performed for the black-pigmented gram-negative anaerobes, including fluorescence testing under long-wave (366-nm) ultraviolet light; hemagglutination of 3% sheep erythrocytes, lactose fermentation by application of the fluorogenic substrate 4-methylumbelliferyl- β -galactosidase (Sigma Chemical Co, St Louis, Mo); and the determination of trypsinlike activity by application of the synthetic fluorogenic peptide.

Results and Discussion

One hundred single root canals were microbiologically sampled from 90 patients. Among these canals, sixty-one teeth had necrotic pulps, and ten of those with necrotic pulps showed radiographic evidence of periapical lesions. Vitality tests showed that these pulps were nonvital. In addition, upon radiographic examination thirty nine teeth were previously endodontically treated, and nineteen of which were poorly obturated canals for more than two years and showed radiographic evidence of apical periodontitis. Forty-one teeth presented coronal restoration, 34 were decayed and 14 were intact. Out of 41 teeth with coronal restoration, 11 teeth were restored with temporary sealing, showing microleakage.

The clinical characteristics of the 100 canals studied were as follows: acute pain (28/100), previous pain (40/100), tenderness to percussion (32/100), swelling (26/100), tooth mobility (6/100), periapical radiolucency (29/100), wet canal (36/100), purulent exudate (21/100), hemorrhagic exudates (8/100) and clear exudates (7/100). Necrotic pulp tissue was observed in 61 canals and the remaining 39 canals

TABLE 1

Prevalence of microbial species in 100 root canals

Microbial species	Root canals		Type of infection	
	Total Number of Microbial Genera	Percentage of root canals	Genera Found in Primary Infected Canals	Genera Found in Secondary Infected Canals
<i>Enterococcus faecalis</i>	19	9.6%	3	16
<i>Peptostreptococcus micros</i>	15	7.6%	13	2
<i>Peptostreptococcus prevotii</i>	18	9.1%	10	8
<i>Streptococcus mitis</i>	14	7.1%	9	5
<i>Streptococcus oralis</i>	4	2.0%	4	0
<i>Streptococcus sanguis</i>	10	5.1%	1	9
<i>Staphylococcus salivarius</i>	17	8.6%	10	7
<i>Bacillus</i> spp	4	2.0%	4	0
<i>Eubacterium lentum</i>	5	2.5%	5	0
<i>Lactobacillus acidophilus</i>	14	7.1%	9	5
<i>Actinomyces odontolyticus</i>	8	4.1%	4	4
<i>Actinomyces naeslundii</i>	4	2.0%	0	4
<i>Actinomyces meyeri</i>	2	1.0%	2	0
<i>Escherichia coli</i>	3	1.5%	3	0
<i>Veillonella</i> spp	5	2.5%	5	0
<i>Porphyromonas endodontalis</i>	18	9.1%	11	7
<i>Porphyromonas gingivalis</i>	6	3.0%	4	2
<i>Prevotella intermedia</i>	10	5.1%	8	2
<i>Prevotella corporis</i>	6	3.0%	4	2
<i>Fusobacterium</i> spp	7	3.6%	7	0
<i>Candida albicans</i>	8	4.1%	8	0

had previously been root filled with gutta-percha.

In the positive culture samples, 20 teeth had a single species, 34 had two species, and 36 had polymicrobial infections consisting of three or more species per canal. Primary endodontic infection, 11 teeth had a single, 20 had two and 27 species had polymicrobial infections consisting of three or more species per canal. No bacteria were recovered in the remaining ten teeth. Individual root canals yielded a maximum of five bacterial species.

A total of 197 cultivable isolates, belonging to 21 different microbial species, were recovered from 100 canals (**Table 1**). Out of 197 isolates, 85 (43.1 %) were identified as obligate anaerobes and 104 (52.8%) as facultative anaerobes and 8

(4.1%) were fungi. Also, according to gram staining characteristic, 55 (27.9%) were gram-negative, 134 (68.0%) were gram-positive species, and 8 were (4.1%) fungi (**Table 2**).

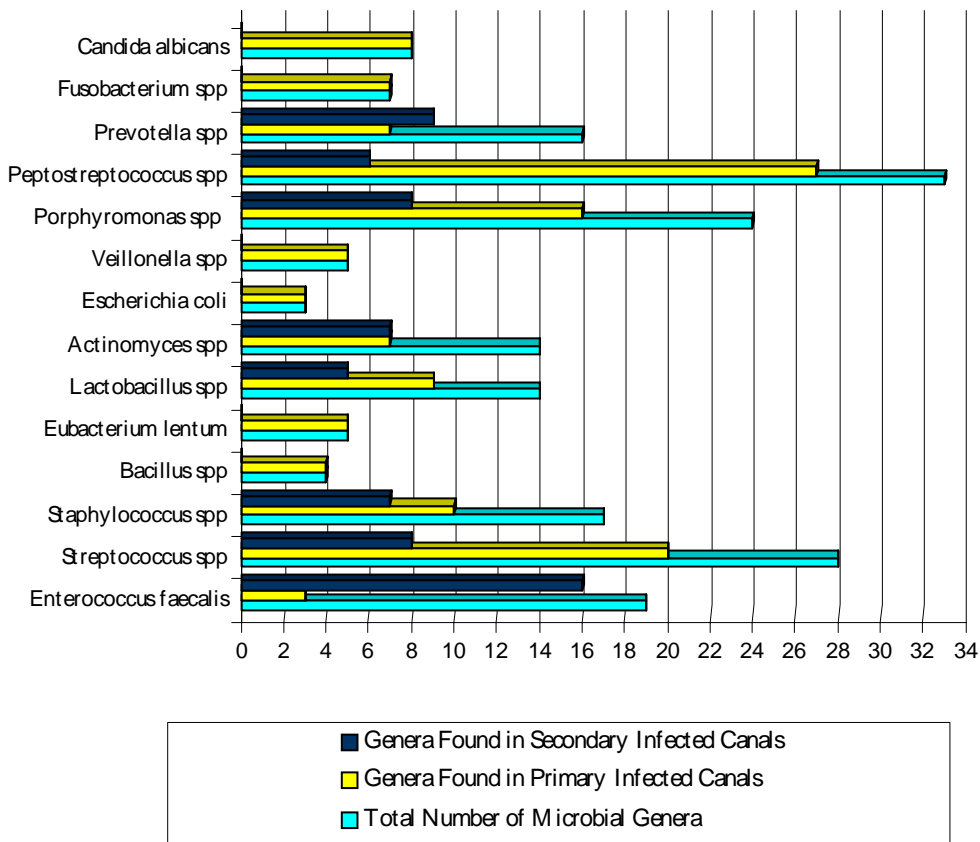
Gram-negative bacteria accounted for 27.9% of total species isolated from the 100 canals. They accounted for 31.2% isolates from primary canal and 23.0% isolates from root-treated canals. Gram-positive bacteria accounted for 68.0% of the total isolates. 62.4% of gram-positive bacteria were isolated from primary necrotic pulp and 77.0% from failed root canal treatment (Table 2).

The prevalence of bacteria and fungi found in 100 root canals is shown in Table 1, while **Figure** shows the prevalence of the microbial genera in primary and secon-

TABLE 2

Prevalence of gram-positives, gram-negative bacteria, obligate anaerobes and facultative anaerobes

	Species found in primary infection		Species found in secondary infection		Total number of species	
	(n)	(%)	(n)	(%)	(n)	(%)
Gram negative	42	(31.2%)	13	(23.0%)	55	(27.9%)
Gram positive	74	(62.4%)	60	(77.0%)	134	(68.0%)
Facultative Anaerobic	55	(44.0%)	49	(68.1%)	104	(52.8%)
Obligate Anaerobic	62	(49.6%)	23	(39.9%)	85	(43.1%)
Fungi	8	(6.4%)	0	0	8	(4.1%)

**Figure.** Prevalence of microbial genera in 100 root canals.

secondary endodontic infection. Peptostreptococcus spp was the most predominantly isolated microbial genera, followed by Streptococcus spp (14.2%), Porphyromonas spp (12.2%), E. faecalis (9.6%), Staphylococcus salivarius (8.6%), Prevo-

tella spp (8.1%), Lactobacillus spp (7.1%), Actinomyces spp (7.1%), Candida albicans (4.1%), Fusobacterium spp (3.6%), Veillonella spp (2.5%), Eubacterium spp (2.5%), Bacillus spp (2.0%), and Escherichia coli (1.6%) were other types of bacteria recove-

red (Figure).

Staphylococcus salivarius, *S. sanguis*, *P. provetti*, *P. endodontalis*, and especially *E. faecalis* were predominant in treated canals. *Streptococcus oralis*, *Eubacterium lentum*, *Fusobacterium*, *Actinomyces meyeri*, *Escherichia coli*, *Veillonella spp* and *Candida albicans* were isolated only from root canal untreated teeth. *Peptostreptococcus provetti*, *A. odontolyticus*, *P. endodontalis* and *S. salivarius* were present in approximately equal proportions in both primary and secondary infected root canals.

In the present study, microorganisms were recovered from 90 out of 100 canals examined. It was not surprising other cultural studies (6, 29, 8, 22) also did not isolate detectable microorganisms in several endodontic samples.

Our study examined the type of microorganisms isolated from primary and secondary infected root canals. Primary root canal infections are caused by microorganisms colonizing the necrotic pulp tissue and impairing its function. The development and spread of pulpal inflammation is related to a number of factors such as caries, trauma, or an iatrogenic cause (28, 21).

Our study showed that the pulp can be infected due to fault restorative procedures, carious lesions, cracking the enamel and via cavity preparation, which frequently opens up extensive dentinal tubular access. All sixty-one teeth had necrotic pulps, and ten of those with necrotic pulps showed radiographic evidence of periapical lesions.

Our findings indicate that amongst the isolates, facultative anaerobic (52.7%) and gram-positive species (67.8 %) were predominant in primary infected root canals, which is confirmed by Sundqvist et al.(22) and Molander et al (8), who found 69% facultative anaerobes and 70% gram-positive microorganisms in their study. Also findings show that the most frequently isolated genera were *Peptostreptococcus* and

Streptococcus. Black-pigmented bacteria such as *Prevotella* and *Porphyromonas* have also been found in primary pulpal lesions. These results are in agreement with previous findings (24, 22, 28)

Secondary infections are caused by microorganisms that were resistant to the chemical-mechanical procedures or invaded the canal during or after endodontic treatment via coronal microleakage (15, 20, 11, 27, 19). In our study, the 39 teeth were previously endodontically treated, and nineteen of which were poorly obturated canals for more than two years and showed radiographic evidence of apical periodontitis.

The most frequently microorganisms isolated from secondary infected root canals were gram-positives. Among them, *E. faecalis* was the species most frequently isolated, agreeing with the findings of Molander et al. (8), Peciuliene et al (13, 14), Pinheiro et al. (15) and Gomes et al. (4). Other bacteria isolated include *S. sanguis*, *S. salivarius*, *L. acidophilus*, *A. odontolyticus*, *P. endodontalis*, *P. gingivalis*, and *P. corporis*.

In conclusion, our results have shown that the flora of infected root canal comprised number of microbial species, predominantly gram-positive ones. Polymicrobial infections and obligate anaerobes were also frequently found in infected canals. The microbiota of infected root canal has polymicrobial characteristics and is predominated by anaerobic gram-positive cocci, which can result in endodontic infections and complaints. Facultative anaerobes, especially *E. faecalis*, were the microorganisms most commonly isolated from secondary infections. The role of anaerobic bacteria in periapical abscesses cannot be overstated; in fact, these bacteria were isolated from 80% of the root canals in this study. These results indicate that new treatment methods should be discovered to eliminate infection during treatment and to improve the prognosis.

REFERENCES

1. **Brown L.R., Rudolph C.E.** (1957) *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.*, **10**, 1094-1099.
2. **Cheung G.S.P., Ho M.W.M.** (2001) *Oral Microbiol. Immunol.*, **16**, 332-335.
3. **Engström B., Frostell G.** (1964) *Acta Odontol. Scand.*, **22**, 43-69.
4. **Gomes B.P.F.A., Drucker D.B., Lilley J.D.** (1994) *Int. Endod. J.*, **27**, 291-298.
5. **Gomes B.P.F.A., Drucker D.B., Lilley J.D.** (1996) *J. Dent.*, **24**, 47-55.
6. **Gomes B.P., Pinheiro E.T., Gade-Neto C.R., Sousa E.L., Ferraz C.C., Zaia A.A., Teixeira F.B., Souza-Filho F.J.** (2004) *Oral Microbiol. Immunol.*, **19**, 71-6.
7. **Kakehashi S., Stanley H.R., Fitzgerald R.J.T.** (1965) *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.*, **20**, 340-349.
8. **Molander A., Reit C., Dahlén G., Kvist T.** (1998) *Int. Endod. J.*, **31**, 1-7.
9. **Möller Å.J.R., Fabricius L., Dahlén G., Öhman A.E., Heyden G.** (1981) *Scand. J. Dent. Res.*, **89**, 475-484.
10. **Möller Å.J.R.** (1966) *Odontol Tidskr.*, **74** (spec issue), 1-380.
11. **Nair P.N.R., Sjögren U., Krey G., Kahnberg K.E., Sundqvist G.** (1990) *J. Endod.*, **16**, 41-49.
12. **Paterson R.C.** (1976) *Br. Dent. J.*, **140**, 231-236.
13. **Peciuline V., Balciuniene I., Eriksen H.M., Haapsalo M.** (2000) *J. Endod.*, **26**, 593-595.
14. **Peciulienė V., Reynaud A.H., Balciuniene I., Haapasalo M.** (2001) *Int. Endod. J.*, **34**, 429-434.
15. **Pinheiro E.T., Gomes B.P.F.A., Ferraz C.C.R., Teixeira F.B., Zaia A.A., Souza-Filho F.J.** (2003) *Oral Microbiol. Immunol.*, **18**, 100-103.
16. **Ray H.A., Trope M.** (1995) *Int. Endod. J.*, **28**, 12-18.
17. **Sato T., Hoshino E., Uematsu H., Noda T.** (1993) *Microb. Ecol. Health Dis.*, **6**, 269-275.
18. **Seltzer S., Farber P.A.** (1994) *Oral Surg. Oral Med. Oral Pathol.*, **78**, 634-645.
19. **Siqueira J.F.** (2001) *Int. Endod. J.*, **34**, 1-10.
20. **Siqueira J.F.** (2002) *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.*, **94**, 281-293.
21. **Siqueira J.F.** (2003) *Int. Endod. J.*, **36**, 453-463.
22. **Sundqvist G., Fidgor D., Sjögren U.** (1998) *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.*, **85**, 86-93.
23. **Sundqvist G., Johansson E., Sjögren U.** (1989) *J. Endod.*, **15**, 13-18.
24. **Sundqvist G.** (1992) *Oral Microbiol. Immunol.*, **7**, 257-262.
25. **Sundqvist G.** (1992) *J. Endod.*, **18**, 427-430.
26. **Torabinejad M., Walton R.E.** (1994) Periradicular lesions. In: *Endodontics*. (J.I. Ingle, L.K. Bakland, Eds.), Baltimore: Williams & Wilkins, 4th ed., p. 439-464.
27. **Tronstad L., Barnett F., Cervone F.** (1990) *Endod. Dent. Traumatol.*, **6**, 73-77.
28. **van Winkelhoff A.J., Carlee A.W., De Graaff J.** (1985) *Infect. Immun.*, **49**, 494-497.
29. **Yoshida M., Fukushima H., Yamamoto K., Ogawa K., Toda T., Sagawa H.** (1987) *J. Endod.*, **13**, 24-28.
29. **Wittgow W.C., Sabiston C.B.** (1975) *J. Endod.*, **1**, 168-171.