

Prevalence of Microorganisms in Root Canals of Human Permanent Teeth with Symptomatic Nonvital Pulp and Chronic Periapical Lesions: A Microbiological Study

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How to cite the article:

Galhotra V, Dey S, Priyank H, Paranjape T, Sharma N, Singh I. Prevalence of Microorganisms in Root Canals of Human Permanent Teeth with Symptomatic Nonvital Pulp and Chronic Periapical Lesions: A Microbiological Study. J Int Oral Health 2015;7(11):71-74.

Abstract:

Aims and Objective: The aim of this study was to evaluate the bacterial profile in root canals of human permanent teeth with necrotic pulp and periapical lesions using microbiological culture techniques to find out the prevalence of various species of microorganisms in these lesions.

Materials and Methods: A 40 root canals of human permanent teeth with necrotic pulp and periapical lesions were examined using bacterial culture. After gaining access to the pulpal tissue, the material was collected using files/broaches for microbiological evaluation and determination of colony forming units.

Results: Anaerobic microorganisms were found in 95% of the samples, black-pigmented bacilli in 37.5%, aerobic microorganisms in 92.5%, streptococci in 95%, and *Streptococcus mutans* in 45%

Conclusion: An infected pulpal tissue of the permanent teeth pulpal and periapical pathologies contain a high number of polymicrobial organisms with the high prevalence of anaerobic *Bacteria* and *Streptococci* species.

Key Words: Anaerobes, microorganisms, periapical abscess, streptococci

Introduction

Various periapical pathologies are often considered as a sequel to infected, untreated, or improperly treated root canals. One of that is apical periodontitis which is usually considered as

an inflammatory reaction in the periapical tissues to various microorganisms within the root canal system.^{1,2} It is obvious and evident that an infected root canal system offers a unique niche and reservoir for the various species of microorganisms.³ Although the qualitative composition of microflora of root canals has been the aim of various research projects over the years, recent literature quotes studies clearly defining the microbial differences between primary endodontic treatment and retreatment.⁴ In patients with pulpal pathology, black-pigmented bacteria (BPB) are frequently been isolated. They have also been implicated in apical abscess formation due to their proteolytic activity.⁵

Apart from endodontic therapy, treatment protocol of acute periapical inflammation includes use of various antimicrobial agents. With the advancement in the research work, the vital role of strict anaerobes in root canal pathologies has been significantly highlighted. Development and isolation of penicillin resistant *Bacteroides* (Beta lactamase producing species) have also highlighted the need for reliable research on antimicrobial susceptibilities.⁶ With time, changes occurring in the species of microorganisms isolated from the pathologies and their antibiotic sensitivity have been identified and pattern of resistance to various antibiotics have been developed.⁷

Hence, the present study was undertaken to study the prevalence of microorganisms from root canals of clinically symptomatic, nonvital teeth with chronic periapical lesions.

Materials and Methods

A total of forty patients with the age group of 25-50 years, of both sexes, seen in the Outpatient department of Index Institute of Dental Sciences Indore, were selected for the study. None of the patient had been treated with antibiotic for at least 3 months. A total of 40 root canals (maxillary and mandibular canines, premolar, and molars) with necrotic pulp and radiographically visible radiolucent areas in the periapical region suggesting chronic periapical lesion were used. Each patient included in study was having at least one intact nonvital tooth, presenting with one or more of the following symptoms:

- Spontaneous pain of varying severity
- Pain on percussion
- Swelling
- Sensitivity to both hot and cold.

Patients with following criteria were excluded:

- Previous endodontic therapy of the affected tooth
- Teeth with bone loss or periodontal pocket more than 3 mm deep
- Teeth with periapical sinus/fistula
- Teeth with calcified canals
- Patients with any generalized disorder or systemic illness
- Patients with antibiotic therapy within previous 2 months.

Sample collection

Total aseptic environment was maintained during the culture sample and culture taking procedure. Rubber dam was placed on the involved tooth. The field was disinfected with tincture of iodine, and the access opening and preparation was done using a sterile round bur. Sterile broach/file was used to collect root canal content for culture medium.

Anaerobic culture

For anaerobic culture, the first reamer/file/broach, entering the root canal up to the apex was dipped in the preheated Stuart’s Transport Medium. The sealed air tight bottle was transferred to the Microbiology Lab of Index Institute of Dental Sciences Indore. Root canal contents were inoculated on Brain Heart Infusion agar and Blood agar supplemented with Anaerobic supplement (Hi-Media) by parallel streak plate method and kept in McIntosh and Fildes Anaerobic Jar at 37°C for 48-72 h. The jar was opened after 48-72 h. Examination of the primary plates was done with the help of hand lens, and each colony type was recorded. Each type of colony was picked up and subcultured aerobically, as well as anaerobically. Organisms failing to grow aerobically were taken as anaerobes.

Aerobic culture

For aerobic culture, after the first broach/file, the next reamer or file containing root canal contents were put in a sterile test tube, sealed and transferred to the microbiology department. Root canal contents were inoculated on Blood agar and MaConkey agar and placed in an incubator at 37°C for 18-24 h. Visualization and examination of the colony characteristics were noted in the case of any growth and Gram-stain and standard biochemical reactions were used for identification of microorganisms.

Results

The colony forming units of the samples from 40 root canals from permanent teeth with necrotic pulp and radiographically visible periapical lesion are shown in Table 1. The prevalence of anaerobic microorganisms was 95% (38 root canals), and BPB were found in 15 cases (37.5%). Aerobic microorganisms were present in 37 root canals (92.5%) with streptococci present in 38 canals (95%). *Streptococcus mutans* was quantified in 18 canals (45%).

Discussion

In the initiation and progression of pulpal and periapical pathologies, the role of bacteria has been well-documented.

Table 1: Prevalence of colony forming units (microorganisms) in root canals of human permanent teeth with non-vital pulp and chronic periapical pathology.

Case	Anaerobic		Aerobic	Streptococci	
	Total	BPB	Total	Total	<i>S. mutans</i>
1	510000	5500	50000	50200	150
2	18000	150	2500	2706	0
3	150	50	256	0	0
4	9000	0	0	8900	0
5	190000	0	535000	235000	75000
6	2000	42	600	290	0
7	0	0	800	55	0
8	210000	0	20000	41000	4520
9	1400	0	120	230	0
10	550	0	90	1500	0
11	2100	45	660	610	0
12	7500	0	1000	0	52
13	45500000	3250000	289000	695000	155000
14	39500000	126000	355000	3540000	212000
15	21900	0	5500	12500	12800
16	2210000	0	189000	780000	0
17	895000	1520	20000	89020	0
18	3156000	290	1200	390	0
19	56000	0	17500	29400	2150
20	3002	0	1100	680	52
21	260	0	50	130	140
22	209000	0	4000	59000	4830
23	2590000	23000	325000	4890000	1320
24	52	0	0	360	0
25	500	0	300	690	0
26	609000	0	89000	69020	142
27	799000	0	10000	59200	0
28	555000	0	65000	292000	690
29	678000	0	255000	157000	140
30	39800000	323000	36000	4560	0
31	802000	0	52000	13200	0
32	610000	5800	55000	52200	150
33	28000	190	2900	2906	0
34	450	70	356	0	0
35	7000	0	0	8700	0
36	290000	0	585000	195000	75000
37	4000	62	900	310	0
38	0	0	600	65	0
39	310000	0	15000	39000	4520
40	2400	0	220	190	0
Total positive cases (%)	38 (95)	15 (37.5)	37 (92.5)	38 (95)	18 (45)

S. mutans: Streptococcus mutans

The success of root canal treatment is directly affected by removal of microorganisms from infected root canals. It is proven fact that the microflora colonizing in the oral cavity can be conducive to pulpal and periapical lesions.^{1,8,9} Experimental studies involving human pulpal pathologies by ter Steeg and van der Hoeven showed that the most important factors involving them are availability of nutrition, oxygen level (redox potential), and the local pH within the root canal.¹⁰

In the present study, anaerobic microorganisms were detected in 95% of the samples. Similar results were also seen in research work reported by Toyoshima *et al.*,¹¹ Sato *et al.*¹² and Pazelli

et al.,¹³ who also found anaerobic bacteria predominance in root canals of human teeth with periapical lesions.

We also found BPB only in 37.5% of the cases of infected pulpal tissue. Similar results were reported by Tomic-Karovic and Jelinek,¹⁴ Faria¹⁵ and Pazelli et al.¹³ in the root canals of deciduous teeth with necrotic pulp. Sundqvist G showed the presence of BPB associated with the formation of abscesses in permanent teeth.¹⁶ Black pigmented Gram-negative anaerobic rods include species formerly known as *Bacteroides melaninogenicus*. These bacteria have been reclassified into two genera: (a) Saccharolytic species - *Prevotella* and (b) asaccharolytic species - *Porphyromonas*.¹⁷

We also found aerobic microorganisms in permanent teeth with necrotic pulp and chronic periapical lesions in 37 root canals. These findings suggest that root canal pathologies are polymicrobial in nature and further squeale depends on the interaction of these various microorganisms.¹³

In our study, streptococci were detected in 38 cases, whereas *S. mutans* was detected only in 18 cases. This was in agreement with the previous studies which showed high frequency of occurrence of *Streptococci* species^{15,18} and lower frequency of the prevalence of occurrence of *S. mutans*.¹⁹

These variations in the prevalence of these microorganisms can be hypothesized in the way that some root canals could have been in direct access to *S. mutans* present in the oral cavity.¹³

Our results show that anaerobic bacteria, BPB, aerobic Bacteria, Streptococci, and mutans streptococci are all components of root canal microorganisms in teeth with pulpal pathology and periapical lesions. All root canals examined in our research consisted of polymicrobial infection with majority of them being anaerobic. These results are in agreement with the previous literature,^{20,21} which shows that CO₂-dependent microaerobic streptococci and other aerobic and facultative microorganisms creates the ideal environment for the growth of anaerobes by consuming and depleting oxygen leading to the development of low redox potential. This leads to the microbial shift and predominance of anaerobic species in the same teeth.²⁰

The treatment of any infection lies in the removal of the etiologic agent. Similarly, the success of root canal therapy relies mostly on the reduction and elimination of bacterial infection.^{22,23} Since past literature quotes that similar microbes occurs in the root canals of primary teeth with necrotic pulp and periapical lesions and in the permanent teeth, endodontic treatment should be similar.

Conclusion

Through our results, we can conclude that the root canals of the permanent teeth pulpal and periapical pathologies contain a

high number of polymicrobial organisms with high prevalence of anaerobic bacteria and streptococci. Further research should be carried out to investigate both qualitative and quantitative root canal microbiota of both permanent and deciduous teeth with necrotic pulp and periapical lesions to improve the prognosis of the treatment therapy.

Acknowledgement

Authors would like to thanks the whole staff of Microbiology department to carry out this study.

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