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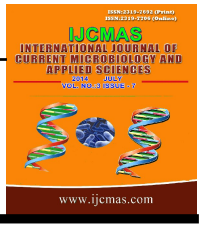


**Original Research Article**

# Microbiolgical analysis of root canal flora of failed pulpectomy in primary teeth

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**A B S T R A C T**

Endodontic treatment of primary teeth with necrotic pulp is routine in dental practice. Control of infection is fundamental because the ample medullary bone spaces favor dissemination of infection and also because the developing permanent tooth germ is very close to the roots of the primary teeth. Information is limited in regard to which microorganisms persist and survive after the completion of root canal therapy. So this study investigated the type of microorganisms in root canals of primary teeth with failed pulpectomies. Samples obtained from root canals of necrotic and failed pulpectomies were cultured in both aerobic and anaerobic media. The development of colonies was studied for gram staining, biochemical reaction and colony characters and micro biota was identified. All the samples showed microbial growth. There was a combination of aerobic and anaerobic microorganisms in primary infected canals and in secondary infected canals, the isolate most commonly recovered were *E. faecalis.*

**Keywords**

Microflora, Infected primary teeth

## Introduction

The primary objective of pulpal treatment is to preserve the integrity and health of the oral tissues (Fuks, 2000). Pulpectomy is indicated in teeth that show evidence of chronic inflammation or necrosis in the radicular pulp, with or without periapical or furcation pathology. The goal of this treatment is to maintain primary teeth that would otherwise be lost. When teeth are treated by root canal therapy under aseptic conditions and according to accepted clinical principles, generally the success rate is high. Most of the clinical studies have reported success rate of 65–100% following this treatment (Chiba, 1981; Holan and Fuks, 1993).

Although many failures are caused by technical problems during treatment, some cases fail even when apparently well treated.

A number of factors have been associated with failure of endodontic therapy, including extraradicular infection, foreign body reactions and true cysts. However, most treatment failures are caused by microorganisms persisting in the apical parts of root canals of obturated teeth (Nair *et al.,* 1990). The clinician is often of the notion that procedural errors, such as broken instruments, perforations, overfilling, under filling, ledges and so on are the direct cause of endodontic failure. In most cases, procedural errors do not jeopardize the outcome of endodontic treatment unless a concomitant infection is present. But, there is potential for failure of root canal treatment when a procedural accident occurs during the treatment of infected teeth.

After determination of the important role of bacteria in the pathogenesis of pulp and periapical lesions (Kakehashi *et al.,* 1965; Tani-Ishii, 1994) elimination of infection from the root canal system became the objective of endodontic treatment of teeth with necrotic pulp and periapical lesions (Marsh and Largent, 1967).

Until 1970, the most common bacterial group isolated by culture from root canals of permanent teeth was viridans *Streptococci* (alpha hemolytic *Streptococci*). Then, with the development of strictly anaerobic culture techniques, the concept of endodontic infection changed because anaerobic microorganisms, which had been rarely isolated, were seen as the predominant endodontic micro biota in permanent teeth with necrotic pulp and periapical lesions. However, there are few studies concerning root canal micro biota of primary teeth.

Marsh and Largent (1967) reported alpha hemolytic *Streptococci* as the predominant micro organisms where as other studies (Cohen, 1960; Tomic-Karovic and Jelinek, 1971) reported *Streptococcus salivarius*. Anaerobic microorganisms represented over 70% of the micro biota in teeth indicated for extraction (Sato, 1990).

Endodontic treatment of primary teeth with necrotic pulp is routine in dental practice. Control of infection is fundamental because the ample medullary bone spaces favor dissemination of infection and also because the developing permanent tooth germ is very close to the roots of the primary teeth. Information is limited in regard to which microorganisms persist and survive after the completion of root canal therapy. This may be a result of the tendency to treat unsuccessful root canal therapy as a technical failure without considering an underlying bacterial cause.

Thus, it is fundamental that the dentist be aware of the micro biota in these teeth so that adequate antimicrobial agents may be used to eliminate these pathogens. The purpose of this study was to investigate the type of microorganisms in root canals of primary teeth with failed pulpectomies and compare it with root canal flora of necrotic primary teeth.

## Materials and Methods

After approval from institutional ethics committee and informed consent from the guardians, 15 patients of both sexes aged 5–9 years were selected from the outpatient department of Pedodontics, Yenepoya Dental College, Mangalore, India.

### Inclusion Criteria

- Healthy children without any systemic illness.
- No Antibiotics received during the last three months.
- Presence of abscess, sinus tract or obvious radiolucency.
- Mandibular second primary molar was selected

**Exclusion Criteria**

- Subjects known to have any systemic illness
- Subjects under antibiotic therapy
- Teeth which could not be isolated
- Teeth less than 2\3 of roots resorbed

Out of 15 patients

- 10 patients had primary teeth with primary infection (necrotic pulp tissues)
- 5 patients had primary teeth with secondary infection (failed endodontic treatment)
- A detailed medical and dental history was obtained from each patient

**Clinical and sampling procedure**

After antisepsis of the oral cavity by rinsing for 1 min with 5 ml of 0.12% chlorhexidine digluconate, local anesthesia was administered, a rubber dam was placed and operative field was disinfected with 1% chlorhexidine digluconate. A two stage acess cavity preparation was performed by employing sterile burs and manual irrigation with sterile saline solution was used instead of water spray.

All coronal restorations and carious lesions were completely removed. The tooth was irrigated with 30% hydrogen peroxide and then with a 2.5% sodium hypochlorite solution for 30 seconds. The solution was inactivated with sterile 5% sodium thiosulfate. Aseptic techniques were used for instrumentation during access to the root canal. In the secondary infected canals; pre existing filling was removed by H file without the use of chemical solvents. Sterile saline solution was irrigated to remove any remaining material and to moisten the canal prior to sample collection absorbent paper points were used for collection from root canal.

In each tooth, samples were obtained from a single root canal to limit the microbiological evaluation to single ecological environment. Two sterile paper points of size compatible with root canal diameter up to the working length or to the level of physiological root resorption and kept in place for 60 seconds one after the other.

The two root canal samples were obtained were transferred immediately in to glucose broth and thioglycolate broth and submitted to microbiological laboratory. Glucose broth was incubated for 24 hours at 37°C and thioglycolate broth was incubated for 48 hours at 37°C. Subcultures were made from brain heart infusion broth in to blood agar and McConkeys media and incubated for 24 hours at 37°C. Subcultures were made from thioglycolate broth in to laked blood agar and neomycin blood agar. These were incubated in anaerobic jar with gas pack for 48 hours at 37°C. The development of colonies was studied for gram staining, biochemical reaction and colony characters.

## Results and Discussion

All the samples showed microbial growth.

**Microorganisms found in primary infected root canals**

- *Klebsiella* 3\10
- *S. viridans* 3\10
- *Enterococci* 1\10
- *Coagulase-ve* 2\10
- *Bacillus spp* 1\10
- *S. aureus* 1\10
- *Peptostreptococcus* 3\10
- *Actinomyces spp* 1\10
- *Fusobacterium spp* 2\10

**Microorganisms found in secondary infected root canals**

- *E. faecalis* 4\5
- *Peptostreptococci* 1\5

Root canal treatment of primary teeth has been controversial, particularly after the publication of the classical study by Hibbard, in 1957. They described the variable and often unpredictable root canal anatomy of primary teeth, as a result of deposition of secondary dentin. Root morphology of primary teeth changes continuously. Continued deposition of dentin will divide in to separate canals which complicate endodontic therapy. This article was widely quoted as evidence that debridement and obturation of the root canal systems of primary teeth was next to impossible, and became the principal deterrent to the development of pulpectomy procedures in the primary dentition. Bacteria can be hidden in these accessory canals, ramifications and apical delta areas. During the 1950s and 1960s, researchers isolated mainly aerobic and facultative bacterial species from root canals with necrotic pulp and periapical lesions due to the limitation of isolation techniques and microbial culture (Shovelton, 1964) With scientific and technological evolution, anaerobic techniques have been developed which showed that root canals of permanent teeth with necrotic pulp and periapical lesions had a polymicrobial infection with predominance of strict anaerobic species (Tronstad, 1992). The micro biota constituted of only a few species when compared to the total bacteria of the oral cavity. There are many factors that can influence the growth and development of these microorganisms in root canals, i.e., nutrient availability, low oxygen tension, bacteria interaction, as well as disintegrated pulp tissue and tissue fluids that are essential nutrient sources (Sundqvist, 1992). In our study in primary infected root canals both anaerobic and aerobic microorganisms, *Streptococci* and Gram-negative aerobic rods were found, but in secondary infected root canals there was a predominance of anaerobic microorganisms. This is in agreement with Toyoshima *et al.,* (1988) who reported that in root canals of primary teeth with necrotic pulp and periapical lesions submitted to retreatment there is a poly microbial infection with predominance of anaerobic microorganisms, similar to the microbiota of permanent teeth (Toyoshima *et al.,* 1988)

Black-pigmented bacilli (BPB) have frequently been isolated from root canals of permanent teeth with necrotic pulp. Sundqvist *et al.,* (1989) reported their presence in 30% of the cases while Assed *et al.,* verified by immune fluorescence that these microorganisms were found in 60% of the samples. Tomic-Karovic and Jelinek (1971) found these microorganisms in 36% of the root canals of deciduous teeth with necrotic pulp. However, Toyoshima *et al.,* (1988) quantified BPB in 44.4% of deciduous root canals in retreatment cases.

This can be due to initial cultivation of sample was made in broth. This increases the likelihood of recovering only the fast growing bacteria when the sample originally contained a mixture of bacterial species. It is also important to note that toxic hydrogen peroxide and superoxide radicals are formed in commercial broth media during autoclaving, which may kill anaerobic bacteria. The literature shows the presence of *Streptococci* in 70%–82% of the root canals of deciduous teeth with pulp necrosis (Marsh and Largent, 1967; Cohen, 1960; Tomic-Karovic and Jelinek, 1971). In our study *Streptococci* was present in 3 root canals. The variations in the prevalence of these microorganisms in different studies can be explained that in some root canals could have been exposed directly to the oral

cavity. *Staphylococci* and *Bacillus* were present in one canal each corroborating with other studies (Marsh and Largent, 1967). This can be due to contamination during endodontic treatment (Reader *et al.,* 1994). This study found that there is a combination of aerobic and anaerobic microorganisms in primary infected canals, although Sato *et al.,* (1993) observed higher prevalence of anaerobic microorganisms over aerobic microorganisms. In secondary infected canals the isolate most commonly recovered was *E. faecalis*. These results show that endodontic infections in deciduous teeth, similarly to those in permanent teeth, are poly microbial with the development of microbial interactions.

Difference in flora between the primary and secondary infected root canals may be due to

- Selective pressure exists in the untreated canal.
- *E. faecalis* can endure prolonged periods of nutritional deprivation
- Altered host response
- Resists intracanal medicaments
- Forms a biofilm

It is concluded that the microbial flora in canals after failed pulpectomies differed markedly from the flora in untreated teeth. It was mainly of a single species of predominantly gram positive organism *Enterococcus faecalis*.

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