

Review Article

Endodontic Microbiology: A Comprehensive Review

Sharik Sharma¹, Manasvi Mahajan²

BDS Intern, GDC Amritsar, Punjab, India

ABSTRACT

Root canal therapy is one of the most commonly employed dental procedures these days. Success of root canal therapy is dependent on the thorough knowledge of root canal anatomy, morphology and microbiology. Hence; we planned the present review to highlight some of the important aspects of endodontic microbiology.

Key words: Endodontic, Microbiology.

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Corresponding Author: Dr. Sharik Sharma, BDS Intern, GDC Amritsar, Punjab, India

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INTRODUCTION

It has been established that bacteria initiate pulpal and periapical infections.¹ The success of endodontic treatment is directly related to the decrease in the number of root canal microorganisms. Colonizing microorganisms result in pulpal and periapical diseases.² Treatment involves the treatment of both primary and secondary infections in the root canal system. Primary infected root canals are untreated canals, into which the microorganisms have gained access to colonize the pulpal tissue, resulting in dysfunction. 4 Secondary infection in the root canal occurs due to the failure of endodontic treatment and presence of bacterial infection in the root canal system.^{3, 4} Several studies have investigated the microflora of root canal system infections.⁴

REVIEW OF LITERATURE

Topcuoglu N et al evaluated the presence of the 10 oral bacterial species in samples from primary tooth root canals by using microarray technology and to determine the association of these organisms with clinical conditions. The samples were collected from 30 root canals of primary teeth with primer infection. The bacterial composition of the samples was semi-quantitatively defined using a microarray system (Parocheck). All the tested species were detected in the samples. *Fusobacterium nucleatum* was the most frequently isolated bacterium (96.7%), followed by *Prevotellaintermedia* (86.7%), *Parvimonasmicra* (83.3%), *Treponemadenticola* (76.7%) and *Tannerella forsythia* (66.7%). These bacteria were also present in high levels.

All pairs of bacterial species were positively associated (RR > 1), except *Pintermedia* and *P >micra*. On average, five species (range:3-8) were detected per amplified sample. Root canals of teeth with > 5 different species were statistically associated with periapical radiolucency (P = 0.049). Primary teeth with endodontic infections show a highly diverse variety of bacteria, in which the most prevalent specie are present in high proportions. The well-directed use of the improved microarray technology will provide additional valuable information for causative factors associated with endodontic diseases, helping to develop more successful antibacterial or anti-inflammatory treatment strategies.⁵

Foschi F et al studied the presence of selected bacteria (*Enterococcus faecalis*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, *Treponema denticola*) in infected root canals using polymerase chain reaction (PCR) assays, and the association of bacteria with clinical signs of endodontic disease was assessed. The null hypothesis, that no difference could be observed between clinical signs of apical periodontitis and a specific bacterial strain, was tested. Microbial samples were obtained from 62 teeth in 54 patients with endodontic disease. For each tooth, clinical data including patient symptoms were collected. Teeth were categorized by diagnosis as having acute apical periodontitis (AAP, teeth with clinical symptoms but no periapical radiolucency, n=22), chronic apical periodontitis (CAP, teeth with radiolucency but no clinical symptoms, n=15) or exacerbated apical periodontitis (EAP, teeth with symptoms and

radiolucency, n=25). Seventy-one percent of cases were primary endodontic infections, and 29% were recurrent ('secondary') endodontic infections (failing cases). PCR assays were used to detect the presence of the selected bacteria. *T. denticola* and *E. faecalis* were each detected in 15 of 62 samples (24%), *P. gingivalis* in 8 samples (13%), *P. intermedia* in 5 samples (8%), and *T. forsythensis* in 4 samples (7%). *T. denticola* was detected in 56% of teeth with EAP. *E. faecalis* was found in 60% of teeth with CAP and in 72% of teeth with secondary infection. Statistical analysis demonstrated an association of CAP and secondary endodontic infection with the presence of *E. faecalis*. ($P < 0.01$). EAP was associated with the presence of *T. denticola* ($P < 0.01$). *T. denticola* was associated with symptomatic endodontic disease in the presence of apical bone resorption.⁶

Gomes BP et al investigated the presence of strict anaerobes such as *Filifactoralocis*, *Tannerella forsythia*, and *Treponemadenticola* in primary and secondary root-infected canals with periapical lesions by molecular analysis and the association of these species with specific endodontic signs and symptoms. Microbial samples were taken from 100 root canals, 50 with necrotic pulp tissues (NPT, primary infection), and 50 with failed endodontic treatment (FET, secondary infection). DNA was extracted from the samples, which were analyzed for the presence of three endodontic pathogens using species-specific primers and PCR. *F. alocis* were isolated from 23 canals with NPT and 12 canals with FET; *T. forsythia* from 12 canals with NPT and three canals with FET; *T. denticola* from 19 canals with NPT and 12 canals with FET. Suggested associations were found between primary infection and the presence of *F. alocis* and *T. forsythia* (both $p < 0.05$). In particular, associations were found between: pain and *F. alocis*; swelling and *F. alocis*; tenderness to percussion and *T. forsythia*; mobility and *T. forsythia* and *T. denticola*; wet canals and *F. alocis*, *T. forsythia*, and *T. denticola*; purulent exudate and *F. alocis*, *T. forsythia* and *T. denticola*; abscess and *F. alocis*, *T. forsythia*, and *T. denticola* (all $p < 0.05$). The findings of this study indicated that *F. alocis*, *T. forsythia*, and *T. denticola* seem to be associated with endodontic signs and symptoms. Additionally, *F. alocis* and *T. forsythia* were detected more frequently in teeth with necrotic pulp than in teeth with failing endodontic treatment.⁷

Gomes BP et al investigated the root canal microbiota of primary and secondary root-infected canals and the association of constituent species with specific endodontic signs and symptoms. Microbial samples were taken from 60 root canals, 41 with necrotic pulp tissues (primary infection) and 19 with failed endodontic treatment (secondary infection). Strict anaerobic techniques were used for serial dilution, plating, incubation and identification. A total of 224 cultivable isolates were recovered belonging to 56 different bacterial species. Individual root canals yielded a maximum of 10 bacterial species. Of the bacterial isolates, 70% were either strict anaerobes or microphilic. The anaerobes most frequently isolated were:

Peptostreptococcus micros (35%), *Fusobacterium necrophorum* (23.3%), *Fusobacterium nucleatum* (11.7%), *Prevotellaintermedia/nigrescens* (16.7%), *Porphyromonas gingivalis* (6.7%) and *Porphyromonas endodontalis* (5%). The root canal microflora of untreated teeth with apical periodontitis was found to be mixed, comprising gram-negative and gram-positive and mostly anaerobic microorganisms and usually containing more than 3 species per canal. On the other hand, facultative anaerobic and gram-positive bacteria predominated in canals with failed endodontic treatment, which harbored 1-2 species per canal. Suggested relationships were found between anaerobes, especially gram-negatives, and the presence or history of pain, tenderness to percussion and swelling ($P < 0.05$). In particular, associations were found between: a) pain (n=29) and *P. micros* ($P < 0.01$), *P. intermedia/nigrescens* and *Eubacterium* spp. (both $P < 0.05$); b) history of pain (n=31) and *P. micros* ($P < 0.01$) *Porphyromonas* and *Fusobacterium* spp. ($P < 0.05$); c) tenderness to percussion (n=29) and *Porphyromonas* spp. ($P < 0.01$), *Peptostreptococcus* and *Fusobacterium* spp. ($P < 0.001$); d) swelling (n=20) and *Peptostreptococcus* spp. ($P < 0.01$), *Porphyromonas* and *Enterococcus* spp. ($P < 0.05$); e) wet canals (n=33) and *Porphyromonas* and *Fusobacterium* spp. ($P < 0.05$); f) purulent exudate (n=20) and *Porphyromonas*, *Peptostreptococcus* and *Fusobacterium* spp. ($P < 0.05$); previous endodontic treatment and *Enterococcus faecalis*, *Streptococcus* spp., *P. micros*, *F. necrophorum* ($P < 0.05$). Their findings indicate potential complex interactions of species resulting in characteristic clinical pictures which cannot be achieved by individual species alone.⁸

PATHWAYS OF PULPAL AND PERIRADICULAR INFECTIONS

The most obvious route for microbial invasion is through an open cavity as a result of dental caries. If the pulp is exposed due to caries, it is exposed to entire oral flora. a-hemolytic *Streptococcus*, *Enterococcus* and *Lactobacillus* are predominantly found with other facultative organisms in smaller numbers.⁹ Where the cementum is missing, dentinal tubules may be the pathway for microbial invasion of the pulp space. Investigators have shown the presence of bacteria within exposed dentinal tubules of both vital and pulpless teeth.¹⁰ However, when a deep carious lesion brings high number of microorganisms to the tubules in proximity to pulp, studies have shown that bacteria will penetrate to the pulp well in advance of the carious process.¹¹ Its contribution as a major source of pulpal infection in the human beings has not been clearly demonstrated. However, it seems possible that anachoresis may be the mechanism by which some traumatized teeth may become infected.¹¹ Pulpal and periradicular contamination through a broken occlusal seal or faulty restoration of a tooth previously treated by endodontic therapy.¹²

ENDODONTIC BIOFILM CHARACTERISTICS OF BIOFILM

The organic substances surround the microorganisms of a biofilm and contain primarily carbohydrates, proteins, and lipids. Among the inorganic elements in biofilms are calcium, phosphorous, magnesium, and fluoride. Bacteria in a biofilm have the ability to survive tough growth and environmental conditions. This unique capacity of bacteria in a biofilm state is due to the following features: Residing bacteria are protected from environmental threats; trapping of nutrients and metabolic cooperation between resident cells of the same species and/or different species is allowed by the biofilm structure. It also exhibits organized internal compartmentalization which helps the bacterial species in each compartment with different growth requirements. By communicating and exchanging genetic materials, these bacterial cells in a biofilm community may acquire new traits. Bacterial biofilm provides a setting for the residing bacterial cells to communicate with each other. Some of these signals, produced by the cells, may be interpreted not just by members of the same species but also by other microbial species. Quorum sensing is process by which communications between these bacterial cells is established through signalling molecules in a biofilm.¹³⁻¹⁵

E. faecalis and yeast, mainly *C. albicans*, has been repeatedly identified as the species most commonly recovered from root canals undergoing retreatment, in cases of failed endodontic therapy and canals with persistent infections. *E. faecalis* are gram positive cocci and facultative anaerobes. They are normal intestinal organisms and may inhabit the oral cavity and gingival sulcus. When this bacterium is present in small numbers, it is easily eliminated; but if it is in large numbers, it is difficult to eradicate. *E. faecalis* has many distinct features which make it an exceptional survivor in the root canal.¹²⁻¹⁵

In post-treatment disease, the microflora is dominated by facultatively anaerobic gram-positive cocci and rods such as *Streptococcus*, *Enterococcus*, *Peptostreptococcus* and *Actinomyces* species. Instrumentation, disinfection and interappointment medication in strict aseptic conditions are essential steps for eradication of microbial species from the infected root canal system. During past decades, *Enterococcus faecalis* and *Candida albicans* have been commonly associated organisms in treatment-resistant infections. Novel microbial detection methods are giving increasing knowledge about microbial species associated with endodontic infections and their roles in them.¹⁵

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