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Review Article

Microflora of Endodontic Infection - A Review

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***ABSTRACT*:**
The aim of endodontic treatment is to identify and eradicate the etiological factor responsible for infection. Thorough Cleaning of the root canalsystem by instrumentation, irrigation and removal of endodontic biofilm is considered as most important factor to prevent and treat the disease. The apical delta, apical fins, isthmuses present in the root canal provide an excellent environment for formation of biofilm and is one of the main cause for reinfection. The following review article explores the role the Endodontic microflora and its role in the success of endodontic therapy .

Key words: Endodontic microflora, Biofilm, Intraradicular infection, Extraradicular infection.

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INTRODUCTION:

The main goal of endodontic therapy depends on preventing the microorganism from infecting and reinfecting the pulp and periapical tissues .Thorough knowledge of understanding the microorganism associated with pulpal disease is important for success of endodontic therapy. (Seltzern*et al*. 1963, Engström*et al*. 1964, Sjögren 1996, Sundqvist*et al* 1998.[1] Abacteriafreeroot canal system is difficult to be obtained due to the anatomical complexities of many root canals, organic residues and unreachable bacteria located deep in the dentinal tubules.[2] This review article explains the role of microorganism and virulence factors in Endodontic infections.

Microbial Factors and its Association with Endodontic Infection

The oral cavity contains around 700 and once the coronal portion of the root canal is been infected, it progresses to the apical area until the bacteria and their products themselves stimulate the periapical tissue leading to apical periodontitis.[3]

The necrotic pulpal tissue in primary endodontic infections has polymicrobial flora with an average of 4-7 intra-canal species, which are often Gram-negative anaerobes .The obligatory anaerobic organisms contribute around 90% of the total bacterial species .The organism within the root canal are present as free floating planktonic i.e single cell or as biofilm attached to the root canal wall .[4]

Table 1: Difference between planktonic cells and biofilm.[5]

Community theory of Infection (Biofilm)	**Germ theory of Infection (planktonic cells)**
Co operating community of various types of microorganism	No community of Microorganisms
Micro organisms arranged in micro colonies Covering by matrix of 'glycocalyx'or 'slime'	Free Living microbial cells
Gradients of pH, Nutrients, and Oxygen tension	No gradients of Nutrients, pH and Oxygen
Increased resistance to antimicrobials	Less resistance to Antibiotics

Intraradicular infection

Microorganisms which colonize within the root canal play an important role in the pathogenesis of periradicular lesions . Kakehashi*et al* (1965) conducted a study were he exposed dental pulps of rat to the oral cavity with microbiota and germ free rats and found that pulp and periapical lesion developed only in conventional rats with oral micrbiota. A study done by Möller*et al* (1981) reported that devitalized infected pulps induced periapical lesion compared to that of uninfected pulp which showed

no periapical lesion. Sundqvist (1976)confirmed the presence of microorganisms and its role in causing periapical lesion and bone destruction. The success of root canal treatment is higher in cases where the infection is removed before the root canal is been obturated .However if the organisms persists within the canal during root filling or penetrate into the canal after obturation due to poor coronal restoration failure of root canal treatment occurs . (Byström*et al* . 1987, Sjögren *et al*. 1997). [1]

Many Studies have shown that the microorganism persist in the apical portion of the root canal even in well treated case . This may be due to the reason that the apical portion of the root canal remain untouched during chemomechanical preparation, regardless of the technique and instruments employed (Lin*et al*. 1991, Siqueira *et al* . 1997). The ability of the microorganism to survive in these conditions is the most critical factor because the organismshas to undergo periods of starvation. Several regulatory factor play an important role for the bacteria to withstand nutrient depletion .Certain genes are activated during the condition of starvation .Eg :Ntr gene system enables bacteria that uses ammonia as a source of nitrogen .[1]

The organisms of intraradicular infections .[6]

1)Black pigmented Gram negative anaerobic rods are the *Bacteroidesmelaninogenicus*.

Taxonomic change of these bacteria include:

(a) Saccharolytic species –*Prevotella* i.e *Prevotella intermedia , Prevotella nigrescens , Prevotellata nnerae, Prevotella denticola.*

(b) Asaccharolytic species – *Porphyromonas* which include *Porphyromonas endodontalis* and *Porphyromonas gingivalis.*

2) The first periodontal pathogen to be detected in endodontic infection is *Tannerella forsythia*

3) *Dialister* species detected in Endodontic infections. eg. *Dialister pneumosintes* and *Dialister invisus.*

4) *Fusobacterium nucleatum, Fusobacterium periodonticum*

5) Spirochetes fall into the genus *Treponema*. The most Prevalent species are *Treponema denticola* , *Treponema sacranskii*

6) Gram positive anaerobic rods of Endodontic infection are *Pseudoramibactera lactolyticus*, *Filifactoralocis*, *Actinomyces spp* ,*Propionibacterium propionicum*, *Olsenella spp*.

7) Gram positive cocci present in Endodontic infection are *Streptococcus anginosus, Streptococcussanguinis, Enterococcus faecalis*.

Extraradicular infection

The spread of microorganism within the body is prevented by the development of periapical lesion which acts as a barrier within the body (Siqueira 1997).The periapical lesion consist of a dense wall of polymorphonuclear leucocytes, or epithelial plug surrounding the apical foramen ,blocking the progression of microorganism .The granulation tissue occupies the space of bone resorption and consist of phagocytes ,antibodies and complement molecules. Very few microorganisms penetrate these barrier but the microbial product like the toxins and virulence factor can diffuse through these defence barrier and cause periapicallesion (Nair 1987). Very few pathogens can advance through such barriers. However, microbial products can diffuse throughthese defence barriers and are able to induce periradicularpathosis.(Tronstad*et a l.* 1987,Tronstad *et al* . 1990, Iwu *et al* 1990, Wayman *et al*.1992, Lomçali *et al*. 1996, Siqueira&Venturim 1997).[1]

The pathogens seen in periapicalpathosisare *Actinomyces*, *Propionibacterium propionicum*, *Treponema spp*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *Treponema forsythia*, *Prevotella spp. And Fusobacterium nucleatum.*[6]

Virulence factors of the microorganism

Lipopolysaccharide (LPS): Endotoxin seen in cell wall of gram negative bacteria.LPS is a bacterial extract free of contamination whereas endotoxins are macromolecular complex of proteins and phospholipids. LPS molecules contain a hydrophilic polysaccharide which are divided into O Polysaccharide specific chain (O antigen) and hydrophobic glycolipid component termed lipid A

The outer surface is made up of lipid A and core is made up of antigen O. The compliment system binds to the long polysaccahride chain of LPS protecting the bacteria from host defence system. This leads to release of proinflammatory and immunosuppressive factors

The biological effect of LPS include

1) Bone resorption- due to activation of monocytes or macrophages which realises pro inflammatory cytokines, prostaglandins and oxygen derived free radicals

2) Increased vascular permeability- due to activation of compliment

3) Production of bradykinin and inflammatory mediator for activation of hageman factor

4) Osteoclast differentiation and bone resorption occurs due to interaction with TLR-4. LPS stimulates rank L expression in osteoblast and secrete interlukin 1,6 and PGE2. The amount of LPS in infected root canal is directly related to the number of gram-ve bacteria especially in cases with symptomatic apical periodontitis

Peptidoglycan: Present in cell wall of gram positive bacteria as 40-100 sheet consisting of complex polymer of glycan portion and a tetrapeptide portion. They activate the macrophages and releases proinflammmatory cytokines such as IL-1beta, IL-6 and TNF-alpha

Lipo Techoic Acid: forms major part of gram negative cell wall .LTA is an anionic polymer of glycerol phosphate covalently bond to glycolipid in cytoplasmic membrane. They release proinflammatory cytokines such as IL-1 beta , IL-6 ,CXCL-8 ,TNF alpha by activation of macrophages all these indirectly causes tissue damage .

Fimbriae: they are found evenly located on the surface of gram negativebacteria which may vary from 10-1000per cell. They originate from the cytoplasmic membrane and their tip mediates bacterial adherence to host tissue or other bacteria.

Capsules/Exopolysaccharide: are highly hydrated water insoluble gel which plays an important role in bacterial pathogenecity and serves as a metabolic substrate during period of starvation.

Extracellular proteins: Stimulates the macrophages, lymphocytes to release proinflammatory and immunomodulatory cytokines, including IL 1, IL6, CXCL 8

Short-chain fatty acids: the obligate anerobic microorganism undergo fermentation and releases butyric acid and propionic acid that causes infection process.

Polyamines: The presence of pain and formation of sinus tract is due to the presence of polycationic molecules like putrescine, cadaverine, spermidine and spermine.

Superoxide anions: these are highly reactive free radical which are produced by bacterial cells and cells of immune system which causes breakdown of the erythrocyte which is involved in interspecies interaction.[7,8]

Review of literature:

Several studies have been performed across the globe detection and eradication of endodontic microflora. We performed a search of PubMed (title search) and google scholar (title search) for peer-reviewed articles that have been published. [TABLE 3]."

Table 2: Virulence factor of microorganisms involved in different stages of infection [9]

Function	Virulence factors
Attachment	Adhesins Outer membrane vesicles Lipoteichoic acid Outer membrane proteins Exopolysaccharides
Invasion	Flagella Dnase collagenase fibrinolysin acid phosphatise
Survival	Exotoxins Heat-shock proteins IgA, IgG, IgM, C3, and C5 Proteinases Exopolysaccharides Metabolic end-products
Direct damage	Exotoxins Hyaluronidase Gingipains Chondroitin sulfatase Aminopeptidases phospholipase, neuraminidase
Indirect damage	Lipopolysaccharide Peptidoglycan Lipoteichoic acid Fimbriae Exopolysaccharides

Conclusion:

Failure of the root canal treatment occurs when the treatment is not of acceptable standards or the treatment is carried out inadequately. In Primary endodontic infection the root canal system provides nutritional supply which favour the growth of anaerobic microorganism. Endodontic failure occurs in persistent or secondary infection this is due to the presence of resistant strains which survive even after antimicrobial treatment. Thus, a better understanding of the characteristics and properties of bacteria and their biofilm along with the environmental changes, is required to enhance success of endodontic therapy.[15]

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Table 3: Characteristics of studies included in the review

AUTHORS	YEAR	AIM	METHODOLOGY	CONCLUSION
Adriana C. Ribeiro, FlaviaMatarazzo	2011	To Explore the Bacterial Diversity in the root canal system using 16S rRNA by cloning and sequencing	Root canal samples of teeth with periapical lesion were collected and 16S rRNA bacterial genomic libraries were fabricated and sequenced to estimate the bacterial diversity within the root canal. A total of 489 clones were analyzed.	Seventy organisms were identified out of which six were new phylotypes Which belonged to the group Ruminococcaceae. The most prevalent taxa were *Dialisterinvisus, Prevotella oris, , Atopobiumrimae, Pseudoramibactera lactolyticus and Tannerella forsythia.* The study concluded that a wide diversity of bacteria were present in the primary endodontic infection , which belong to family Firmicutes, class Clostridia followed by the phylum Bacteroidetes.[10]
H. J. Rolph, A. Lennon	2001	Molecular Identification of Microorganisms from Endodontic Infections	41 clinical Samples from 15 primary case and 26 refractory cases of endodontic infections were analysed. 44% of cases showed positive for culture and 68% of cases were positive for PCR.	The study showed the presence of *Enterococcus, Lactobacillus, Propionibacterium*, and *Streptococcus in two cases*, primary case showed the presence of genera *Lactobacillus, Pantoea, Prevotella*, and*Selenomonas*. The five refractory cases showed the presence of genera *Capnocytophaga, Cytophaga, Dialister, Eubacterium, Fusobacterium, Gemella, Mogibacterium, Peptostreptococcus, Prevotella, Propionibacterium, Selenomonas, Solobacterium, Streptococcus*, and *Veillonella*[11]
Anuradha Rani, , Ashok Chopra	2011	Isolation and identification of root canal bacteria from symptomatic non vital teeth with periapical pathosis	Total sample size-30	Out of total 30 samples, 26 showed positive cultured with 69 isolates. 5 teeth showed pure anaerobic organism , 5 purely aerobic and 16 had mixed growth of anaerobic and aerobic isolates. [12]
Blome B, Braun A	2008	The aim of the study was to determine the total bacterial count and the presence and no.of species specific bacteria in patients with chronic apical periodontitis using quantitative real-time polymerase chain reaction (q-PCR).	Total patient included in the study was 40 with 20 denovo cases and 20 with reinfection. After the initial step of access opening and removal of necrotic tissue the first bacterial sample was collected. Following chemomechanical preparation of the root canal a second sample was collected .A 14 days of intracanal dressing was given with calcium hydroxide and third sample was collected. Real-time PCR was used to quantify the total bacterial count	The results of the study showed the presence of P. micros ,along with *P. endodontalis, P. gingivalis, F. nucleatum, and T.Forsythia.*[13]
Ashraf F. Fouad, Jody Barry	2002	Identification of Bacteria Associated with Endodontic Infections using PCR Technique	The total of 24 microbial sample with necrotic pulp were included in the study .10 putative bacterial pathogens with necrotic pulp were identified using primers that target the bacterial 16S rRNA genes .The role of these organisms and their association with diabetes mellitus were also analysed .	The results of the study showed the presence of *Fusobacterium nucleatum* and *Porphyromonas gingivalis* and the presence of *P.gingivalis* and *Porphyromonas endodontalis* in patients with diabetes mellitus.[14]

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