Microbial Changes in Endodontic Infections

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***Abstract*: The objective of endodontic treatment is to prevent the sequelae of apical periodontitis and create a favorable environment for healing of peri radicular tissues. The rationale for endodontic treatment is to eliminate the previous infection or to prevent microorganisms from reinfecting the root canal and peri radicular tissues. Understanding the complex microbiologic etiologic facets and evidence-based management forms the basis of a successful endodontic treatment. This article highlights the microbial aspect of endodontic infections, the pathogenicity, and its management.**

***Index Terms*: Microorganism, Biofilm, Bacteria, Endodontic infections**

I. INTRODUCTION

Infection of the root canal leading to apical periodontitis is termed as an endodontic infection (1). Microorganisms are responsible for the progression and perpetuation of endodontic infections, even though various physical and chemical factors induce periradicular inflammation (2).

Antony van Leeuwenhoek (1632-1723) was the first person to record the presence of bacteria in the root canal. He observed that the root canal of a rotten tooth was "filled with a soft substance" and that it looked to be alive and termed it "Animalcules"(3). In 1894, Willoughby Dayton Miller, analyzed samples from root canal systems and observed an association between bacteria and apical periodontitis. He found that the bacteria were present in the morphologic forms of cocci, bacilli, and spirilla (or spirochetes). (Fig. 1) Most of the bacteria were conceivably anaerobic bacteria and could not be cultured due to lack of technology. The microbiota present in the coronal, middle and apical thirds of the root canal, differed in morphology. Miller proposed a hypothesis that bacteria were the etiological agents of apical periodontitis based on his findings (4).

After 70 years Miller's hypothesis was confirmed by Kakehashi and his colleagues (2).Kakehashi et al performed a study in conventional germ-free rats and observed the response of exposed dental pulps to the oral cavity. Histologic evaluation revealed necrosis of the pulp and apical periodontitis. The exposed pulps healed with hard tissue formation and separated from the oral cavity again.

A similar study by Sundqvist confirmed the significant role of bacteria in the causation of apical periodontitis. Apical periodontitis cannot be induced or sustained by necrotic pulp and stagnant tissue fluid in the absence of infection (5). He also confirmed the microbial causation by demonstrating that apical periodontitis was induced only by infected devitalized pulps and that there were no significant changes induced by non-infected devitalized pulps. He and his colleagues also demonstrated that the induction and maintenance of apical periodontitis can be done by the necrotic pulp tissue as such (6). Apical periodontitis caused by microorganisms are present in the biofilms of the root canals. This was first observed by Nair who stated that they “resemble biofilm structures” (7). Ricucci and Siqueira proved that the primary and post treatment apical periodontitis were associated with a higher prevalence of bacterial biofilms (8).

Pathways of Infection: The ways in which microorganisms reach the pulp are as follows (9):

Dentinal tubules: Dentinal tubule provides an unobstructed passage for bacteria to reach the pulp. Bacterial invasion into the tubules is faster with a nonvital pulp compared with a vital pulp (10).

Factors influencing dentin permeability and delayed intratubular invasion by bacteria:

- Movement of dental fluid and tubular contents in an outward direction (anatomical diameter of tubules is only 5% to 10% of physiological diameter) (11),
- fibrinogen deposition in the intratubular area
- dentinal sclerosis below a carious lesion,
- smear layer,
- tertiary dentin (12),
- host defense molecules like antibodies and complement system components present in the dentinal fluid (13).

Only when there is significant reduction of dentin thickness and permeability, the vital pulp provides a pathway for infection. However, if there is a compromise in the vitality of the pulp with an impaired defense mechanism, even a negligible number of bacteria can induce infection (14).

Open cavity: Traumatic exposure or iatrogenic error can result in exposure of the pulp leading to a direct contact with the septic oral environment (9).

Periodontal membrane: The lateral channel or the apical foramen acts as a pathway for the microorganisms that pass through the periodontal membrane from the gingival sulcus (9).

Blood stream: After trauma or an operative procedure, the inflammation produced without pulpal exposure causes bacteria present in the blood to be attracted to the pulp. Bacterial attraction via blood or lymph is called as anachoresis, serves as a pathway for endodontic infection (9).

Faulty restoration: Bacteria can gain access to the periapical tissue and cause infection if there is a broken temporary restoration or a fractured tooth before final restoration, or if there is an inadequate final restoration (9). Salivary contamination from the occlusal aspect can reach the periapical tissues in less than 6 weeks in root canals obturated with gutta percha and sealer (15).
Enamel crack: Macro and microcracks in enamel, sometimes they can extend deep into the dentin following trauma. If pulp becomes necrotic due to traumatic exposure, they lose their ability to protect against bacterial invasion (16).
Microbial penetration into after initiation of root canal therapy: Dental biofilm residues, calculus, dental caries, leaking rubber dam, contamination of endodontic instruments and irrigation solutions or other solutions used for intracanal cleaning and shaping (saline solution, distilled water, citric acid) can cause penetration of microbes. Microbial leakage can occur in between dental appointments due to fracture, breakage of temporary restoration or the tooth structure, teeth that are left open for drainage. Fracture and destruction of the temporary or permanent restoration can serve as a pathway for microbial penetration after the completion of root canal (14).
Pulp necrosis and periapical pathology development
Pulp necrosis is an irreversible condition that occurs when the soft pulp tissue within the tooth dies. Pulp necrosis can follow either pulpitis or a traumatic injury to the apical blood vessels cutting off the blood supply to the pulp. A coagulative type of necrosis is seen after ischemia. If the necrosis follows pulpitis, then the breakdown of inflammatory cells may lead to the liquefactive type of necrosis which may become infected by bacteria from caries (14).Sequelae of pulp necrosis and periapical pathology development (Fig. 2) (17)

1. Progression of the carious lesion with a breakdown of enamel and dentin barriers.
2. Pulp inflammation, in which the first line of pulp defense occurs with the migration of innate immune response cells.
3. Pulp necrosis, from which inflammation of the pulp develops.
4. Periapical lesions, in which pulp necrosis shifts the immune response to the periapical region.
5. Immune-inflammatory response in a periapical region with innate and adaptive cells and products.
6. Bone resorption, initiated and maintained by RANKL (Receptor Activator of Nuclear Factor Kappa B Ligand) which signals the initiation of both osteoclastogenesis and activation of osteoclasts, thereby influencing bone resorption.

MECHANISMS OF MICROBIAL PATHOGENICITY

Pathogenicity refers to the ability of a microorganism to cause infection. Microorganisms that cause disease in a given host routinely, are reoffered to as the primary pathogens. Opportunistic pathogens cause disease only when the host defenses are impaired.
In caries lesions, the bacteria form the authentic biofilms adhere to the dentin. Even before the pulp is exposed, bacteria penetrate through the tubules and induce pulpal inflammation. After pulpal contact, caries colonizes and covers the bacteria in the biofilm. The exposed pulp tissue then comes in direct contact with the microorganisms and their products leading to severe inflammation. Bacteria on the battlefield have to survive the attack from host defenses as well as obtain nutrients to survive themselves and are called early root canal colonizers or pioneer species. These early colonizers are responsible for initiating the apical periodontitis disease process that significantly modifies the environment, making it suitable for the establishment of other bacteria. In this bacterium-pulp collision, the latter is always "defeated" and becomes necrotic, so the bacteria "occupy the area" move towards the apical portion of the root canal until almost the entire canal becomes necrotic and infected. Newer species (late colonizers) may have access to the canal through coronal exposure or the tubules of the exposed teeth (14).
Although colonization may appear to be an easy task for late colonists, other environmental factors (for example, interactions with pioneer species, oxygen tension, nutrient availability) will determine whether new species entering the canal establish themselves and join the early colonists to form a dynamic mixed community in the root canal. There can be rearrangement in the proportions of the pioneer species and latecomers. Few early colonizers do not participate in the consortium of advanced disease and become more spatially and structurally organized (17). Bacteria that colonize the necrotic root canals cause damage to the surrounding peri radicular tissues leading to inflammatory changes. These changes can occur before the frontline of infection reaches the apical foramen (18).
Virulence specifies the degree of pathogenicity of a microorganism to cause a disease. Virulence factors include the microbial products (secreted products, including enzymes, exotoxins, heat shock proteins, and metabolic end products), structural cellular components, or strategies (coaggregation and biofilm formation) that contribute to pathogenicity. Through direct and indirect pathogenic mechanisms, bacteria can cause destruction of the host tissues.

DIRECT MECHANISM: Bacterial virulence factors that cause direct tissue damage are the damaging host cells or the intercellular matrix component of the connective tissue (17). Lipopolysaccharide (LPS)/ endotoxin is an integral part of the cell wall of Gram-negative bacteria. It is associated with pulpal pain, inflammation in the periapical tissues, activation of complement components, and destruction of the periapical bone (19). Proinflammatory and anti-inflammatory cytokines is upregulated by peptidoglycan in the T cells (20). Lipoteichoic acid (LTA) is a component of Gram-positive bacterial call wall. It is composed of echoic acid and lipid that activates complement cascade and causes damage (21). Fimbriae helps in adhesion to surfaces and interactions with other bacteria. Capsules facilitate the protection of the bacterial cell against desiccation, phagocytosis (9). Extracellular vesicles involved in release of bacterial products into the extracellular environment that is involved in hemolysis, hemagglutination, adhesion of bacteria, and proteolytic activities (22). Proteases are extracellular proteins produced by the bacteria. They neutralize immunoglobulins and complement components and enzymes such as, chondroitin sulphatase, hyaluronate lyase, beta-glucuronidase, DNase, and acid phosphatase. These enzymes cause tissue disintegration (23).

INDIRECT MECHANISM: Bacterial components stimulated by inflammatory and non-inflammatory host cells release chemical mediators such as cytokines and prostaglandins which induce bone resorption in an asymptomatic chronic apical periodontic lesions (14). The formation of oxygen-derived free radicals (superoxide and hydrogen peroxide) accompanied by the release of lysosomal

enzymes by polymorphonuclear leukocytes causes the destruction of the connective extracellular matrix, leading to the formation of pus causing acute apical abscess (24).

SPATIAL DISTRIBUTION OF THE MICROBIOTA

In primary infections, root canal microbiota is predominated by bacterial cocci, rods, filaments, and spirilla (spirochetes). (Fig.3) Fungal cells are infrequently found (25).

In the main root canal, planktonic bacterial cells which are enmeshed in the necrotic pulp tissue and suspended in a fluid phase can be observed. These microorganisms can easily be accessed and eliminated during treatment. Most of the bacteria that colonize the root canal system typically develop in sessile multispecies biofilms that adhere to the walls of teeth. Bacteria can also be clogged in the lateral canals, apical ramifications, and isthmuses that connect the main canals. These organisms are more difficult to gain access to and necessitate special therapeutic strategies for their elimination (25).

Bacterial cells (frequently dividing cells) from the endodontic biofilms are frequently observed penetrating the dentinal tubules in 70% to 80% of the teeth exhibiting apical periodontitis lesions (26). A shallow penetration is more common, but bacterial cells can be observed reaching approximately 300 µm in some teeth. Bacteria can acquire nutrients within tubules through the degrading odontoblastic processes, denatured collagen, bacterial cells that die during the course of infection, and intracanal fluids that enter the tubules by capillarity (25). Bacteria that can penetrating the dentinal tubules include Actinomyces israelii , Porphyromonas endodontalis, Fusobacterium nucleatum Porphyromonas gingivalis,Propioniba acnes, Enterococcus faecalis, Candida albicans, and streptococci (27).

BIOFILM AND BACTERIAL INTERACTIONS

Biofilm is defined as a sessile multicellular microbial community that is characterized by cells that are firmly attached to a surface and enclosed within a self-produced matrix of extracellular polymeric substance (EPS), which is usually a polysaccharide (28).

Classification of Endodontic bacterial biofilms:

- Intracanal biofilms
- Extra radicular biofilms
- Biomaterial centered infections
- Periapical biofilms

Intracanal Microbial Biofilms: These biofilms form on the root dentin in an infected tooth. It is observed as a palisade structure with extracellular matrix material similar to dental plaque visible on the tooth surface.

Extraradicular biofilm: They are root surface biofilms which form on the root surface adjacent to the root apex of an endodontically infected teeth. Based on 16s rRNA gene assay, Fusobacterium nucleatum, Tannerella and Porphyromonas gingivalis, were found to be associated with extra radicular biofilms (30)

Periapical biofilm: They are present peri apically as isolated biofilms and can be seen even when there are no infections of the root canal system. It is associated with Actinomyces species and Propionibacterium propionicum. Their presence is influenced by pH, ion strength, and cell concentration which is promote biofilm formation (31).

Foreign body centered biofilm: They are found when bacteria adhere to an artificial biomaterial surface. It is also called as biomaterial-centered infection. Takemura et al observed that the formation of extracellular polymeric matrix surrounding gutta-percha is influenced by gram-positive facultative anaerobes. Serum plays an important role in formation of such biofilms (32).

Basic criteria for a biofilm

Caldwell et al highlighted few characteristics of biofilm which are as follows:

- Autopoiesis -The ability to self-organization which must be possessed by microorganisms.
- Homeostasis -Should resist environmental perturbations
- Synergy -Rather than in isolation, they should be more effective in the community.
- Communality -Responding to environmental changes as a unit and not individually (33).

Bacteria in biofilm form microcolonies (15% by volume), arise from surface colonization by planktonic (unattached) bacterial cells (34) which are separated by open water channels that pass through the biofilm matrix. It creates primitive circulatory systems (35). Extracellular matrix constitutes 85% of the biofilm volume. It is primarily composed of polysaccharides, proteins and nucleic acids (36). Genes expressed by cells in biofilms vary between 20% and 70% of similar cells grown in planktonic culture (37). Cell-cell communication (quorum sensing) in a biofilm through signaling molecules, coordinate gene expression. When a variety of factors including Ph, osmolarity, oxygen stress, density of the cell is exposed to the microcolonies, they create a unique and diverse microenvironment in the biofilm structure (14).

RESISTANCE TO ANTIMICROBIAL AGENTS

Bacteria arranged in biofilms are known to be more resistant to antibiotics than similar cells developed in planktonic stages. The concentration of antibiotics required to kill the bacteria in the biofilm is hundred to thousand times higher than the amount needed to kill the same species in the planktonic stage (38).

MECHANISMS

1. Biofilm structure may restrict the entry of antimicrobial agents

Cells located deep in the biofilm may remain relatively unaffected. It contains neutralizing enzymes in the matrix at concentrations that can cause inactivation of the antimicrobial agents (39).

2. Altered growth rate of biofilm bacteria

Most antibiotics need at least a certain amount of cellular activity to be effective. Bacteria grow slowly in conditions of low nutrient availability in an established biofilm which is consequently much less susceptible than rapidly dividing cells. Therefore, bacterial cells in the stationary phase may represent antibiotic resistance in biofilms (38).

3. Presence of "persister" bacteria

Few specialized cells can increase the antibiotic resistance of the biofilms. These cells are called “persister” cells (40).

DIVERSITY OF THE ENDODONTIC MICROBIOTA

The oral cavity contains the maximum aggregates of microorganisms in the body with bacteria being the most predominant. However, viruses, fungi, protozoa and archaea are also found. About 10 billion bacterial cells are present in the mouth cavity. More than 50% to 60% of the oral microbiota are still cultivated and fully characterized (41). Endodontic infection occurs in a previously sterile space. The endodontic bacteria are categorized into 9 phyla based on modern molecular techniques

- Firmicutes,
- Spirochaetes
- Bacteroidetes,
- Actinobacteria
- Fusobacteria,
- Proteobacteria,
- Synergistats,
- TM7, and
- SR11 (14)

Microbial Ecology and Root Canal Ecosystem:

Different species of bacteria predominate at different stages of root canal infection. In the initial stages of the pulpal infectious process, alternative bacteria predominate (41). After a few days or weeks, the root canal becomes depleted of oxygen due to loss of circulation in necrotic pulp and oxygen consumption by facultative bacteria which leads to growth of anaerobic bacteria (14). Due to the bacterial byproducts and its metabolites, different oxygen pressure gradient is created in the root canal system. Consequently, the microbiota in different parts may also differ in the diversity, density, and accessibility of treatment processes (14), (Fig.4).

Bacterial Interactions

Positive interaction which enhances the survival capacity of the interacting bacteria. Positive interactions are coaggregation, interbacterial nutritional interactions (Fig.5) decreased oxygen tension in the environment favors anaerobes that causes release in some proteinases which provide protection from host defense. Negative interactions which act as feedback mechanisms that limit population densities. Negative interactions are competition for nutrients and space (14).

TYPES OF ENDODONTIC INFECTIONS

- Intraradicular infection
- Primary infection
- Secondary infection
- Persistent infection
- Extraradicular infection

PRIMARY INTRARADICULAR INFECTION

Initial or “virgin” infection is caused by microorganisms that cause initial invasion and colonization of necrotic pulp tissue in untreated teeth. It is the cause of primary apical periodontitis. Mixed community conspicuously dominated by anaerobic bacteria. The number of bacterial cells can range from 103 to 108 per root canal. An average of 1020 species/phylotype can be present per infected canal (42). Sinus tract associated root canals may display a species number close to the top of this range. The presence of bacterial species and the number of cells in the root canal is proportional to the apical periodontitis lesion size[42].The prevalence of certain species differs according to different geographical locations (43).

Other microorganisms in endodontic infections: Candida species, which are present in the oral cavity are only infrequently detected in primary intraradicular infections (44). Methanobrevibacter oralis–like phylotype, an archaea , have been observed in primarily infected canals[45]. Human immunodeficiency virus (HIV) has been observed in vital pulps of HIV seropositive patients.Herpesviruses has been isolated from inflamed and noninflamed vital pulp (46). Human cytomegalovirus (HCMV) and Epstein Barr virus (EBV) have been isolated from apical periodontitis lesions (47).

PERSISTENT/SECONDARY INFECTIONS AND TREATMENT FAILURE

The major causes for endodontic treatment failure include secondary intraradicular infection and persistent intraradicular infections caused by antimicrobial resistant microorganisms (9,14).

Bacteria at the Root Obturation Stage:

Meticulous antimicrobial treatment may not completely remove the bacteria from the infected root canals because they are either inaccessible or resistant to the treatment (14). The most common cause of primary infections are gram negative bacteria, and are usually eliminated. However, gram positive bacteria are more resistant to antimicrobial treatments and adapt to harsh environmental conditions in instrumented and medicated root canals. Bacteria that persist in the root canal after chemo-mechanical procedures or intracanal therapy may not sustain an infectious process (48).

Few apical periodontitis lesions have been healed even after bacteria were isolated from the canal at the obturation stage because the residual bacteria may die due to the toxic effects of the filling material. The virulence and presence of the residual bacteria is essential in maintenance and persistence of peri radicular inflammation where there is no instrument access (49).

Microbiota in Endodontically Treated Teeth

Only 1-5 species can be isolated from root canals which are well-treated. However, inadequately treated root canal systems can harbor to 10 to 20 species, which is equivalent to the number of species present in untreated canals. Root canal systems with posttreatment disease harbor 103 to 107 species per canal (49). E. Faecalis is the most commonly isolated species in root canal treated teeth and are frequently recovered from teeth treated at multiple visits or teeth which are left open for drainage (50,51). E.faecalis, escape from instrumentation and irrigants since they are present in the deeper parts of the dentinal tubules (52). Resistant of E.facecalis to calcium hydroxide is due to a proton pump that moves the protons into the cell to make the cytoplasm acidic (53).

E. faecalis may enter a "viable but not cultivable" state (VBNC)where they lose their ability to grow in culture media but the viability and pathogenicity is maintained. However, division resumes when the environmental conditions are optimal (54).

E. faecalis as a major causal agent of endodontic failure has been questioned by some studies. Although, they are easily cultivated, E. faecalis is not detected in all studies which evaluate the microbiota of posttreatment disease root canal systems (55). E. faecalis is rarely isolated from retreatment cases and have been more frequently isolated from teeth with no lesions rather than root-canal treated teeth (56).

EXTRARADICULAR INFECTIONS

Extra radicular infection is the infection of the peri radicular region. It can be dependent or independent of intraradicular infections. The main causes of extra radicular infections are few bacterial species that overcome host defenses and are aggregated near or beyond the apical foramen. An example of extra radicular infection independent of intraradicular infections is apical actinomycosis (57,58). Species involved in the extrardicular infections include Actinomyces, Propionibacterium and Fusobacterium (59).

MICROBES IN ENDODONTIC FLAREUPS

Flareups can be pain, swelling, or both and represent an acute emergency (60). Chavez de Paz examined root canal microbiota and revealed that F. nucleatum is associated with flareup. Other microbes involved in flareups are Prevotella and Prophyromonas (Black pigmented bacteria), and Bacteroides melaninogenicus (61).

METHODS FOR MICROBIAL IDENTIFICATION

The endodontic microbiota has been identified by either microbiologic culture methods or molecular biology methods. Culture is the process of propagation of microorganisms in the laboratory by providing the optimum physical and chemical environment for growth (61). The steps include sample collection and transportation, dispersion, dilution, culture, isolation, and identification. Limitations are the impossibility of culturing a large number of existing bacterial species, not all viable bacteria can be isolated, difficulty in isolation techniques and a greater time duration required for isolation of anaerobes.

Molecular biology-based techniques have overcome these limitations and don't require cultivation. Molecular techniques reliably identify cultivated bacteria, rare isolates, new species and poorly described or obsolete bacteria (14).

The choice of a particular molecular technique depends on the microorganism to be isolated[62]. Some of the techniques available include:

- Broadrange PCR - used to know the breadth of microbial diversity
- Gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (tRFLP)-used to analyze the bacterial community.
- Pyrosequencing and Fluorescence in situ hybridization (FISH)- used to identify structure of bacterial communities
- DNA–DNA hybridization arrays, species specific single PCR, nested PCR, multiplex PCR, and quantitative realtime PCR-used to detect a specific microorganism in a clinical sample.

The advantages of different microbial detection methodologies are given in a Table 1. (63-65)

CONCLUSION

Among, the different microorganisms present, bacteria are the most predominantly isolated from pulpal and periapical infections. The advancements in the molecular techniques show the evidence of the association between microbes and endodontic infections. Therefore, to attain success in the management of endodontic infections, a thorough knowledge of the microbes involved in the infection and its sequelae is necessary.

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Figure 1: Drawings from Miller showing different bacterial forms in a root canal sample

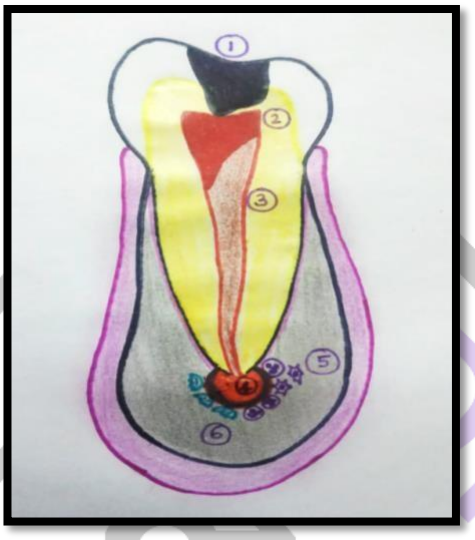

Figure 2 Schematic diagram of pulp and periapical pathology development

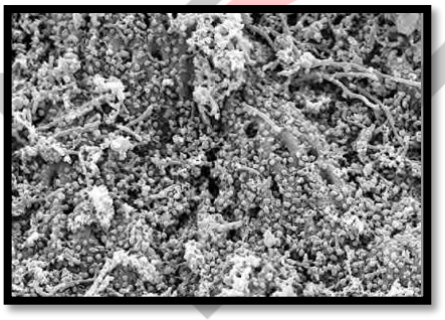

Figure 3 Mixed bacterial population colonize the root canal wall. Cocci is the most prevalent form, but rods, filaments, and spirochetes are also present

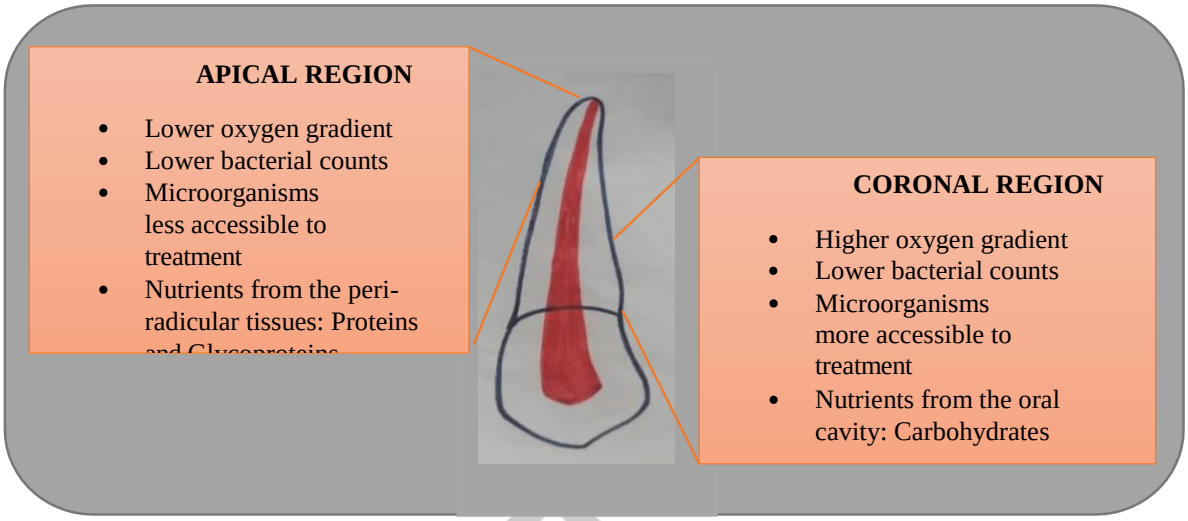

Figure 4 Ecological conditions on different parts of the root canal

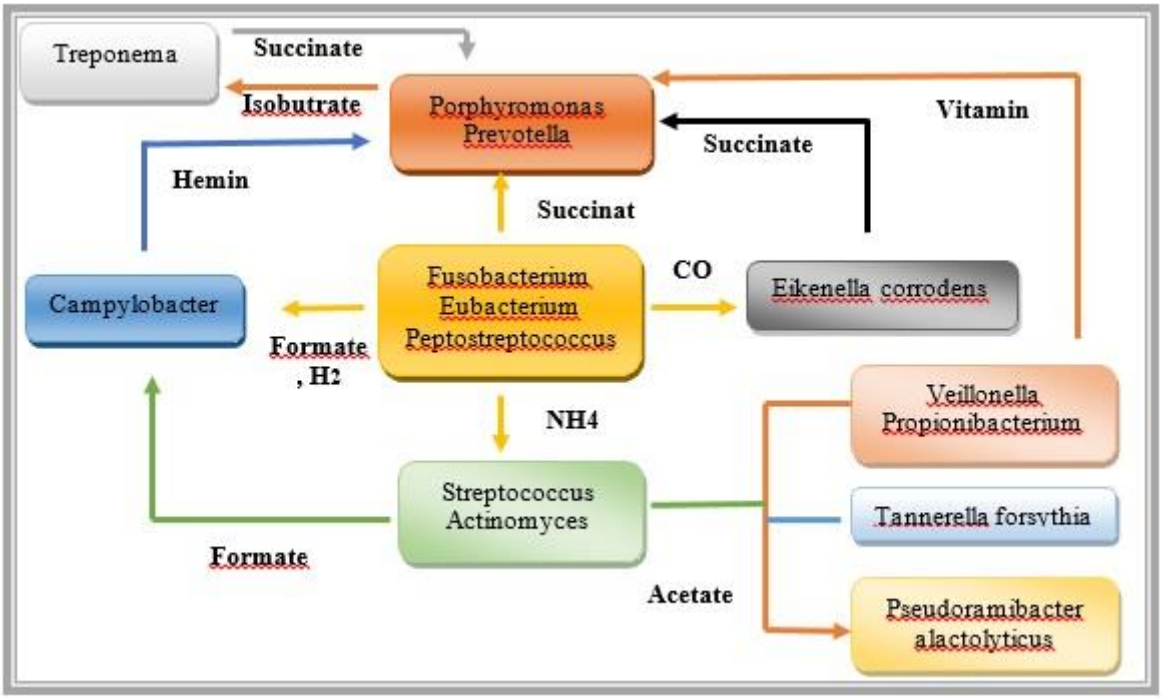

Figure 5 Interbacterial nutritional flow chart

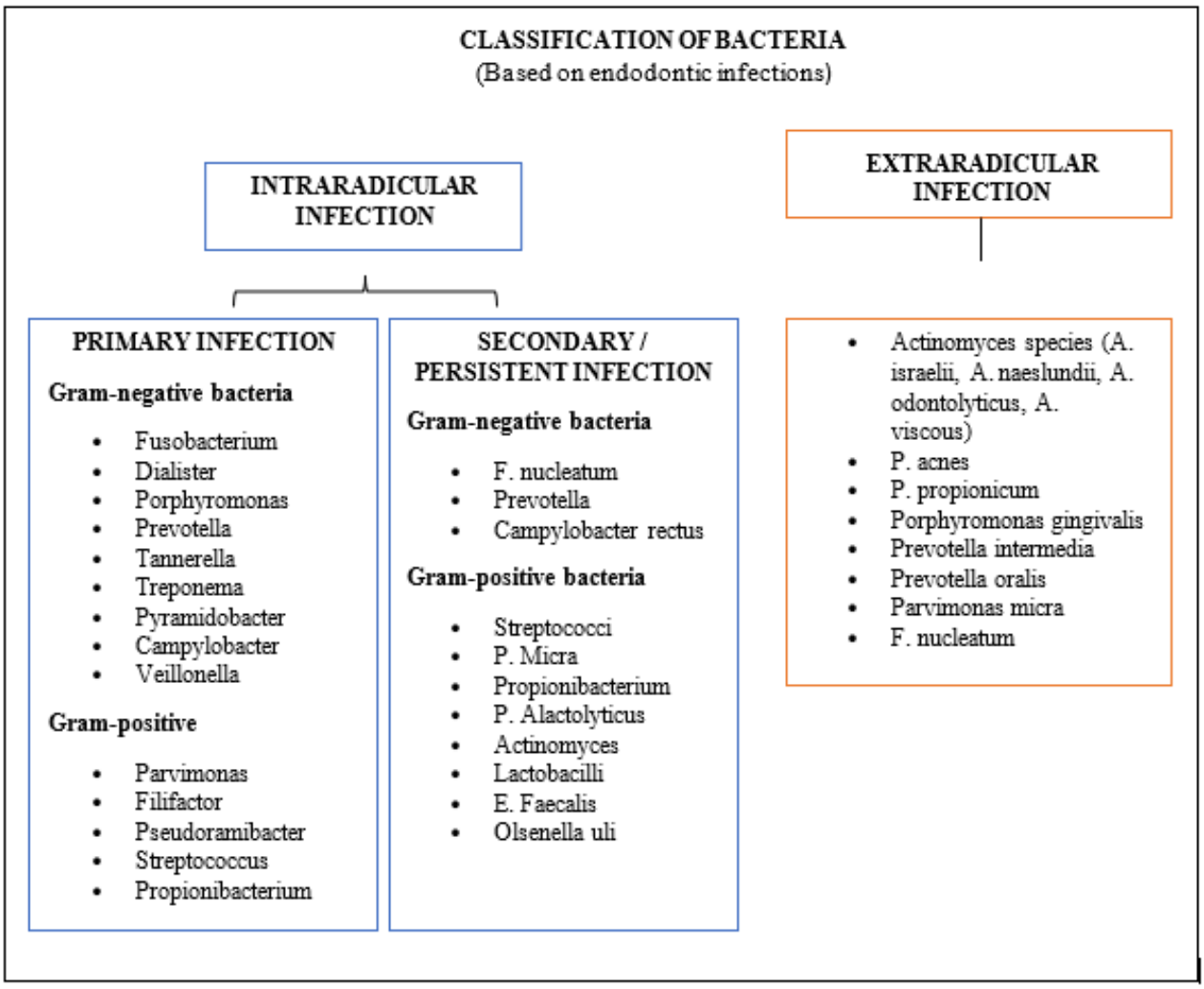

Figure 6 Classification of bacteria based on endodontic infections

TABLE 1: Main advantages of different microbial detection methodologies

CULTURING	MOLECULAR METHODS
Allows study of microbial virulence	High sensitivity of microbial identification
Allows testing of antibiotic resistance	Precise taxonomic classification of microorganisms, identification of pathogenic strains, and relative abundance of different taxa
Allows in vitro testing and experimentation.	Precise study of microbial virulence, interactions, and gene expression
Easily identifies bacterial load (number) comprehensive analysis of protein expression; Estimation of bacterial load	Comprehensive analysis of protein expression; Estimation of bacterial load
Shows microbial viability	Viability can be confirmed by the detection of mRNA.