Microbiology in endodontics: A review

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Abstract

Root canal system acts as an ‘affluent sanctuary’ for the growth and survival of endodontic microbiota. This is attributed to the special environment which the microbes get inside the root canals. Although variety of microbes such as viruses and fungi have been isolated from the root canal system, bacteria are the most commonly found to be associated with Endodontic infections. These are the first to reach the pulp and initiate the pulpal reaction through direct pulp exposure, lateral/accessory canals or through periodontal membrane and blood stream. The oral cavity is home to multiple anatomic micro niches which may vary from birth to old age due to changes in oral environment however root canal being the closed space harbours different microbiota including gram negative and positive microorganisms. To achieve the best results with root canal therapy it becomes important to have knowledge about the residing microbiota and how to combat them.

Keywords: microbes, endodontics, root canal, infection

Introduction

Endodontics is that branch of dentistry that is concerned with the morphology, physiology and pathology of the human dental pulp and periapical tissues. Improvement in the success of endodontics is due to the complete understanding of the microbiology involved in the endodontic pathology. The rationale behind this is the complete extermination of the root canal infection, to prevent micro-organisms from infecting or re-infecting the root and periapical tissues. Endodontic infections are different from the other oral infections in the fact that they occur in an environment which is closed to begin with since the root canal system is a confined one, surrounded by hard tissues all around. For endodontic infection to develop, the root canal must be devoid of vital pulp tissue and its defenses, as an outcome of either pulp necrosis or pulp removal for the treatment. Once necrotic, the RCS becomes a “privileged sanctuary” for bacterial clusters, their by-products, and degradation products of both the microorganisms and the pulpal tissue [1].

Dental caries is the most common cause of pulp injury. The bacteria in the front of the carious process are the first to reach the pulp and initiate the pulpal reaction. In addition to caries, pathways for the entry of microorganisms into the pulp space include direct pulp exposure (e.g. trauma, and dental procedures), dentinal tubules, lateral/accessory/furcation canals, anachoresis, enamel cracks, invasion through periodontal membrane and blood stream [2].

The microorganisms colonize the root canal system from the oral cavity after ecological selection. The contribution of bacteria to these infections has been widely established and had been seen as pulpal or periapical disease based on their clinical diagnosis. Pulpal diseases could be reversible or irreversible pulptitis, pulp degeneration or pulp necrosis that mainly includes gram positive microbes and anaerobes in predominant numbers whereas periapical diseases involves periodontitis, apical abscess, cysts and granuloma with majority of them involving poly microbial communities of gram positive facultative anaerobes. Microorganisms from other kingdoms, such as viruses, bacteriophages, archaea and fungi has also been examined [3]. Fungi are highly prevalent and diverse within the healthy oral cavity and often associated with oral disease. They can be found within root canal infections as well and their contribution to periapical disease might be significant.
In the last 15 years, there has been growing interest in studying the presence of different viruses in periapical lesions. Most of these studies have analyzed the association between the presence of viruses in apical periodontitis and their correlation with clinical signs and symptoms. Ferreira D et al. (2011) conducted a study to detect herpesviruses types 1 to 8, as well as human papillomavirus (HPV) in acute apical abscesses and it was found that a large number of abscess samples were positive for at least one target virus.

Normal oral microbiota & ecosystem
The oral cavity is a unique ecosystem that is aseptic at birth with infant mouth being sterile at birth, except perhaps for a few organisms acquired from the mother’s birth canal (vertical transmission). Oral flora on the child’s first birthday usually consists of streptococci, staphylococci, Neisseria, together with some gram-negative anaerobes such as Veillonella spp. The next evolutionary change in this community occurs during and after tooth eruption where Gram-positive and gram-negative organisms colonize the enamel and crevicular tissues. During puberty, variation in hormone levels also alter the oral microbiome, that leads to transition to an adult flora. Spirochaetes, Veillonella, Prevotella, and black-pigmented Bacteroides are more frequently isolated during this period of life. As age advances, the direct and indirect effects of senility affect the microbial homeostasis where the last major oral bacterial alteration is reached if all teeth are lost. Arweiler L. et al. (2016) studied the interaction between normal oral microbiota and described that normal oral flora is polymicrobial in nature and has an important function to protect against colonization of extrinsic bacteria which could affect systemic health.

Endodontic microbiome
The main goal of endodontic therapy is the thorough mechanical and chemical cleaning of the entire pulp cavity and its complete obturation with an inert filling material. Miller was the first who described the presence of a characteristic root canal microbiota. It was also seen that the variation in the nutrient and oxygen tension in the apical region compared to that of the main canal are the causative factors for the growth of obligate anaerobes at the apical site. The synergistic mechanisms between the various endodontic pathogens involve an interaction of numerous factors, like providing nutrition, inhibition of phagocytosis secretion of growth factors and enzymes, decrease in the local oxygen concentration and oxidation–reduction potential and local pH in the root canal. These mechanisms enhance the survival and pathogenesis of obligate and facultative anaerobes.

When the established caries remain untreated, they increase in width and depth. The dentine is clinically exposed and the biofilm is heavily involving the cavity. As the deep lesion further progresses the color of the demineralized dentine becomes darker and at the central area the cariogenic biofilms overlying the lesion are markedly reduced with decrease in oxygen reduction potential. Progressive alterations of pulp inflammation can be observed via action of gram positive bacteria through lipoteichoic acid that releases pro-inflammatory cytokines eventually accounting for 70% of the cultivable microbiota. S. mutans is the main organism that adheres to the canal wall and initiates the process however Lactobacillus selectively aggregates into the retentive niches formed by the S. mutans that produces acid, inhibits nonaciduric bacterium, and degrades dentinal collagen to promote the development of caries. Gram-negative bacteria will take over as the lesion advances, and, because of their lipopolysaccharide content, they are capable of inducing lipopolysaccharide–binding protein which has an even more complex role in terms of triggering the pulpal inflammation which progresses to irreversibility and further pulpal necrosis, if left untreated. This degeneration occurs gradually from coronal to apical direction as the environment is more anaerobic and nutrient supply is abundant from periapical tissues that favors the bacterial growth.

Pathogenicity refers to the ability of an organism to cause disease in another organism. These organisms are known as pathogens which include bacteria, fungi, viruses, protozoa and parasites. Their ability to induce direct tissue damage could be due to enzyme induction, exotoxins and metabolites whereas indirect tissue damage can be induced from a host immune reaction that has the ability to cause tissue destruction, stimulated by bacterial components which include lipopolysaccharide (LPS), peptidoglycan (PG) and lipoteichoic acid (LTA). The degree of pathogenicity or disease producing ability of a microorganism is known as virulence. Identification of specific virulence factors present in bacteria might play a role in inhibition of endodontic infections.

Pathways of pulpal invasion
According to Narayanan and Vaishnavi (2010), the various routes by which the microorganisms reach the pulp could be: Enamel cracks: After a carious lesion or during dental procedures, microorganisms may use the pathway in a centripetal direction to reach the pulp through dentinal tubules.

Open cavity: Direct pulp exposure of traumatic origin such as in coronal fracture, or that of iatrogenic nature, breaks the physical barrier imposed by dental structures and leaves pulp in contact with the septic oral environment.

Periodontal membrane: Microorganisms from gingival sulcus may reach the pulp chamber through the periodontal membrane, using a lateral channel through lateral canal or through apical foramen.

Blood stream (Anachoresis): The bacteria present in the blood would be attracted to the dental pulp following trauma or operative procedure that produced inflammation without causing pulp exposure.

Faulty restoration: It has been documented that salivary contamination from the occlusal aspect can reach the...
periapical area in less than 6 weeks in canals obturated with gutta percha and sealer.

**Microbiology of endodontic infections**
Microbiology of endodontic infections can be studied under the following criteria:

A. **Types of endodontic infection on the basis of occurrence**[1]
   - Primary endodontic infection: Bacterial genera frequently detected in primary infections are:
     1. Gram-negative bacteria (Fusobacterium, Dialister, Porphyromonas, Prevotella, Tannerella, Treponema, Campylobacter, and Veillonella)
     2. Gram-positive bacteria (Parvimonas, Filifactor, Pseudoramibacter, Olsenella, Actinomyces, Peptostreptococcus, Streptococcus, Propionibacterium, and Eubacterium)
   - Secondary/Persistent endodontic infection: Gram-positive species and facultative anaerobes have a higher rate of recovery in post-instrumentation samples like Streptococcus species, Parvomonas micra, Actinomyces spp., Propionibacterium spp., Pseudoramibacter alactolyticus, Lactobacillus spp.

B. **On the basis of location**
   - Intraradicular infection: The root canal flora of teeth with clinically intact crowns, but having necrotic pulps and diseased periapex is dominated by obligate anaerobes, usually belonging to the genera Fusobacterium, Porphyromonas, Prevotella, Eubacterium, and Peptostreptococcus
   - Extraradicular infection: The extraradicular infections are dependent or independent of an intraradicular infection. The dominant microorganisms present are anaerobic bacteria like: Actinomyces spp., Propionibacterium propionicum, Treponema spp., Porphyromonas endodontalis, Porphyromonas gingivalis, Treponema forsythia, Prevotella spp., Fusobacterium nucleatum.

C. **On the basis of clinical diagnosis**
   - Pulp diseases
   - Periapical diseases

**Classification of pulp diseases can be discussed as According to Grossman**

I) Pulpitis
   - a) Reversible
     1. Acute (symptomatic)
     2. Chronic (asymptomatic)
   - b) Irreversible
     1. Acute
     2. Chronic

II) Pulp Degeneration
   - Calcific (Radiographic diagnosis)
   - Others (Histopathologic diagnosis)

III) Pulp Necrosis
   - Reversible Pulpitis: Reversible pulpitis is a mild-to-moderate inflammatory condition of the pulp caused by noxious stimuli that resolves with removal of the irritant. This happens when bacteria are closer to pulp but still confined to primary/secondary dentin that allows for reversibility of pulpal inflammation once stimulus is removed. The reason for this is the presence of cariogenic environment that may maintain high acidogenic domain for a longer period before the undermined enamel gradually breaks down.
   - Response to EPT occurs at lower current levels indicating a lowered pain threshold. Cold and hot stimuli produce different pain responses in normal pulp. When heat is applied to teeth with un-inflamed pulp, there is a delayed initial response[10].

**Microbiological analysis**: The understanding of bacterial amount in reversible pulpitis is important to establish a clinical protocol of treatment. It has been commonly seen and documented that reversible pulpitis is predominated by Firmicutes and Actinobacteria genera amongst which the former was mostly represented by Lactobacillus, Pseudoramibacter, and Streptococcus, whereas the most common representative genera of the latter were Propionibacterium, Olsenella and Actinomyces. This is because of the fact that Streptococcus and Lactobacillus had better adhesive capability to form a retentive niche characterized by low pH and anaerobic conditions conducive to their growth. (Sun C et al.[11])

Caneppele TFM et al. (2020) conducted a study to determine bacterial diversity and endotoxin levels in deep carious lesions of teeth presenting symptoms of reversible pulpitis and found out that symptomatic teeth showed greater presence of Lactobacillus species, Capnocytophaga sp., and Leptotrichia buccalis with higher prevalence of endotoxins in asymptomatic cases.

**Irreversible Pulpitis**: In this condition, the pulp has been damaged beyond repair, and even with removal of the irritant it will not heal. The pulps will progressively degenerate, causing necrosis and reactive destruction. Progression of the inflammatory process to the stage of acute abscess denotes an irreversible pulp condition.

**Microbiological analysis**: Microorganisms mainly bacteria present in the advanced front of carious dentin can be considered as etiologically significant in the development of pulpitis. The most commonly detected microbes in irreversible pulpitis are gram positive rods such as lactobacillus, streptococcus but in addition to this few gram negative anaerobes like Prevotella species had also been found. The increased growth of these organisms was seen due to the increase in glycoprotein content in inflammatory exudate found in irreversible pulpitis. (Martin F et al.[13], Zheng J et al.[14])

Zahran S et al. (2021) determined the presence of bacteria within pulps presenting with clinical symptoms of irreversible pulpitis using molecular methods and found that the most abundant genera observed were Veillonella (16%), Streptococcus (13%), Corynebacterium (10%), Cutibacterium (9.3%), and Porphyromonas (5.7%)

Zheng et al. (2019) conducted a study to identify the microbiota of deep dentinal caries that progresses from reversible pulpitis to irreversible pulpitis and found that in the pulp with deep caries and reversible pulpitis, both Firmicutes and Actinobacteria were highly abundant whereas in symptomatic irreversible pulpitis, the abundance of Firmicutes was the highest. These results might indicate that Actinobacteria decreases and Firmicutes increases as carious
lesion progresses.

**Pulp Necrosis:** Necrosis, death of the pulp, may result from an untreated irreversible pulpitis or may occur immediately after a traumatic injury that disrupts pulpal blood supply.

**Microbiological analysis:** Complete degeneration of pulp involves the presence of obligate and facultative anaerobes amongst which most frequently found genera were of gram negative rods (Prevotella, Porphyromonas) and gram positive cocci (Streptococcus and Peptostreptococcus). Their presence was attributed to the reason of low oxygen tension and high nutrient availability due to exposed pulp in case of pulp necrosis (Chu F et al. [16], Fabris A et al. [17]).

Cavalcante AM et al. (2018) [18] conducted a study to analyze the type of microbiota present inside the necrotic root canals through the colored Gram method. 30 root canals were analyzed with absorbent paper cones which were then inoculated in a Brain Heart Infusion Agar (BHI) culture medium and incubated in an oven for 48 h at 37°C. It was seen that all the root canals with pulp necrosis and periapical lesions showed Gram-negative micro-organisms(100%), whereas in the canals of 23 teeth, it was possible to find cocci and Gram-positive bacilli (95.8%). They concluded that there was no statistically significant difference between the amount of Gram-positive and negative bacteria in endodontic infections, indicating that the microbiota of these endodontic infections is mixed.

**Periapical diseases**

Nair in 1997 classify periapical diseases as follows

1. Acute apical periodontitis
2. Chronic apical periodontitis
3. Apical abscess – acute or chronic
4. Periapical cyst – true or pocket

**Acute & Chronic Apical Periodontitis (AAP)**

- The first extension of the pulpal inflammation into the periradicular tissues is called acute apical periodontitis (AAP). The patient may give a history of pain due to previous pulpitis. When periodontitis develops, exudates escape into the periodontal membrane causes the tooth to be extruded by a minute amount and the bite to fall more heavily on it. The tooth is at first uncomfortable, then increasingly tender, even to mere touch.

- Chronic apical periodontitis (CAP) results from pulpal necrosis and usually is a sequel to AAP. It is characterized by a slightly widened apical periodontal space containing diluted blood vessels, mild inflammatory exudates, and a dense accumulation of chronic inflammatory cells.

- Microbiological analysis: Apical Periodontitis involves majority of the strains of gram positive and gram negative facultative anaerobes that includes Porphyromonas species, Fusobacterium, Actinomyces and Streptococcus. This mixed diversity was seen due to different physicochemical conditions and type of nutrient availability in apical third of canals with apical periodontitis. (Lee LW et al. [19], Siqueira JF et al. [20])

- Vengerfeldt V et al. (2014) [21] conducted a study that aimed to reveal root canal microbial communities in cases of chronic apical periodontitis using Illumina sequencing and it was found that highly polymicrobial communities were present in apical periodontitis amongst which the most numerous were Firmicutes and Bacteroidetes, but Actinobacteria, Fusobacteria, Proteobacteria, Spirochaetes, Tenericutes, and Synergistetes were also present. Bouillaguet S et al. (2018) [22] characterized the microbiota present in primary and secondary intraradicular infections associated with apical periodontitis using 16S rRNA gene amplicon sequencing. PCR amplicons of the bacterial 16S rRNA gene were pooled & sequenced and clustering of reads into operational taxonomic units (OTUs) was done. It was seen that the most prevalent and abundant OTU in both dentin and root canal samples were anaerobic bacteria, Fusobacterium nucleatum.

**Apical Abscess**

- An abscess is a localized collection of pus in a cavity formed by the disintegration of tissues. Acute (symptomatic) apical abscess (SSA) is a localized or diffuse liquefaction lesion that leads to destruction of periradicular tissues; an inflammatory response to microbial and nonbacterial irritants from necrotic pulp.

- Microbiological analysis: Collection of pus becomes a favorable environment for the growth of anaerobic bacteria that includes gram negative anaerobes like Fusobacterium, Prevotella and Porphyromonas. Detection of few facultative and Gram-positive bacteria (Streptococcus and Peptostreptococcus) provide heterogeneity to microbial culture in case of an abscess. This mixed consortium is due to the presence of interbacterial nutritional interaction that results in higher metabolic efficiency and ability to grow in anaerobic environment (Siqueira JF et al. [23], George N et al. [24]).

- Zhang W et al. (2020) [25] conducted a study to detect the predominant bacteria in acute periapical abscesses using culture-independent molecular methods based on 16S ribosomal DNA cloning and it was found that the most dominant genera were Streptococcus (13.3%), Fusobacterium (11.8%), Parvimonas (7.8%), Prevotella (6.7%), Sphingomonas (5.8%), and Hafnia (5.2%). Fusobacterium nucleatum (11.5%), Parvimonas micra (7.8%), Streptococcus intermedius (6.6%), Sphingomonas echinosides (5.3%), Hafnia alvei (5.2%), and Citrobacter freundii (4.9%) were the most common species/phyotypes.

**Periapical Cyst (Radicular Cyst)**

- The cysts are of two categories the bay cysts or pocket cysts and the true cysts. Bay cysts are those in continuation with the root canal and true cysts are usually detached from the root canal. Most of the periapical cysts are symptomless and are discovered when periapical radiographs are taken of teeth with non-vital pulps.

- Microbiological analysis: Periapical cyst incorporates heterogeneous group of gram-positive facultative anaerobic bacteria that includes streptococcus species and Actinomyces israelii, usually found in symbiosis with the human microbiota of dental plaque, it is because of their ability to adhere to intracanal surfaces and ability to act as secondary colonizers that led to the progression of periapical lesion to a cyst. (Gomes NR et al. [26])

- Tek M et al. (2013) [27] performed a study to detect predominant bacteria associated with radicular cysts and found out that microorganisms isolated from cultures were Streptococcus milleri Group (SMG) (23.8%), Streptococcus sanguis (14.3%), Streptococcus mitis (4.7%), Streptococcus
Viruses in endodontic pathosis

Viruses are the simplest and smallest microorganisms that infect humans. They consist of either DNA or RNA surrounded by a protein coat termed a capsid. The scientific study of the viruses and viral infection began at the end of the 19th century.

The most commonly known viruses within oral cavity are the herpes viruses, DNA viruses that cause oral disease in humans. In past two decades, new viruses have been identified that have expanded our knowledge and understanding of viral infections. Human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV) seem to be important putative pathogens of human periodontitis and symptomatic periapical lesions, causing pathosis either by inducing immunosuppression or by infecting periodontal cells directly. Viral association with bacteria and apical disease is consistent with pathologic role of both infectious agents which has been explained by various studies.

Li Hong et al. (2009) [28] identified the presence of herpes viruses, including human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV-1), and Varicella zoster virus (VZV) in patients with irreversible pulpitis or apical periodontitis, either primary or previously treated.

Makino K et al. (2015) [29] conducted a study to find the etiology of chronically inflamed periapical granuloma using molecular methods like PCR. Sample was collected from 32 periapical granulomas and EBV-DNA was found to be present in 25 of the samples. It was concluded that EBV could play a pivotal role in controlling immune cell responses in periapical granulomas.

Fungi in endodontic pathosis

Unlike bacteria, fungi are chemoorganotroph eukaryotic microorganisms that may be found in 2 basic forms: molds and yeasts. Molds are multicellular filamentous fungi consisting of branching cylindrical tubules whereas yeasts are unicellular fungi, and cells are spherical or oval in shape. C. albicans, one of the well-studied fungal species, can reproduce by budding, which results in the formation of yeast cells. Their ability to survive as commensals in diverse, anatomically distinct body sites, allows them to grow in the neutral pH of the bloodstream.

Microbiological analysis: Fungi are considered to be more prevalent in persistent root canal infections and reinfections that have communication with the oral cavity and in immunocompromised individuals.

Egan MW et al. (2002) [30] conducted a study to determine the relative prevalence and diversity of yeasts in salivary and root canal samples from the same patients and they found that yeasts like C. albicans and C. sake, Rodotorula mucilaginosa occurred relatively infrequently (10%) in root canals.

Persoon IF et al. (2017) [31] systematically review the literature on the prevalence and diversity of the fungi found in endodontic infections in the combined 54 studies that included a total of 3603 participants and overall prevalence of fungi in root canal infections was found to be 7.5% amongst which Candida albicans was the most frequently isolated species.

Conclusion

Microorganisms play an unequivocal role in infecting root canal system. Endodontic infections are different from the other oral infections in the fact that they occur in an enclosed environment, surrounded by hard tissues all around. The endodontic infections constitute almost 40-50% of the overall oral diseases. Several bacteria along with fungi and viruses can be identified depending on the health status of the tooth (caries, pulpitis, necrotic tooth, acute/chronic periodontitis, and persistent intra/extra radicular infections). Hence, it was concluded that role of knowing microbiology in endodontic infections provide an aid to the cure of the disease.

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