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Review of microbiology of endodontics and molecular identification of microorganisms from endodontic infections

Abstract

Endodontic therapy aims at eliminating the existing infection and also inhibiting re-infection. Endodontic failures attribute almost 30-40% of dental failures. Microorganisms are recognized worldwide as the etiological agent for the majority of endodontic infections. The current day medicine emphasizes on understanding the etio-pathogenesis of disease process rather than just symptomatic approach. The limitations of conventional culture techniques like low sensitivity, non-specificity etc has paved way for more potential molecular biology techniques. The molecular techniques are highly sensitive & highly specific making it an integral part of modern day medicine and research. This tool gives insight to the interactions of molecules like DNA, RNA and proteins that are the key factors for life process. A significant contribution of molecular biology methods to medical microbiology relates to the identification of previously unknown human pathogens. More than 50 % of previously uncultivable oral bacteria have been identified by this advanced technique. The techniques can be applied extensively for diagnosis, management and prevention of pathology. This review intends to familiarize various molecular techniques and underlying concepts. This in turn facilitates in implementing them to various contemporary areas of research.

Keywords: Microorganisms, endodontic infection, root canal, endodontic pathogens, molecular biology

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Introduction

A new era of clinical medicine has emerged highlighting the significance of diagnostic microbiology in the disease process. The awakened interest in the biomedical community over the last several years for the study of microorganisms is substantial. Accurate identification of microbial isolates is paramount in clinical microbiology. The microflora of oral cavity is diverse and distinct as it harbors wide variety of bacteria, fungi, archae and virus.

The micro-flora comprising the root canal is typically unique and specific. The endodontic infections constitute almost 40—50% of the overall oral diseases. Pulpal and periapical pathology are the commonest debilitating form of oral disease with systemic implications. Though success rate of endodontic therapy ranges from 30-90% of treated cases, failure rate is equally high accounting to millions. The blossoming advanced technological aids have probably detracted our attention from primary problem of endodontic disease.

The comprehension of the microbial location, characteristics and behavior in the root canal assists in decoding the disease process. However, for impromptu treatment outcomes, there is a need for this data to be translated into clinical practice. The dominance of certain species in some locations and geography-related pattern occurrence has been confirmed by Community-profiling. Most of the current knowledge of endodontic taxonomy is based on international reports. The geographical variation, ethnicity, food habits, oral hygiene and many other local factors can influence the type of flora and their behavior. Hence it is significant to establish the microbial profile of the local population to deliver specific targeted therapy.

The negative effect of systemic diseases on oral microflora is well established. The altered environment caused by systemic diseases influences the community virulence and interferes with the treatment results. The chance of more pathogenic microbial profile in medically compromised patients explains the trend toward increased painful apical periodontitis, flare-ups and decreased success.

The culture technique has been the standard method of studying microflora in infectious diseases. If pathogens are uncultivable, detailed characterization of few species and also population, molecular (genomic) analysis can be an excellent tool. In endodontics, many are proteomic analyses but limited genomic studies. But molecular analyses have few inadequacies in envisaging physiology, function and pathogenicity of the disease and host factors. In-spite of limitations of culture technique, they are still excellent choice for their broad range approach and phenotypic characterization. Hence the combination of both the techniques can contribute immensely in microbial analysis.

Although the incidence of virulence factors is more pronounced in hospital infections, endodontic isolates are exhibiting emergence of bacterial resistance to conventional endodontic regimens. The different expression profiles of virulence can be explained by geographic differences, diet, infection stage and systemic conditions. These parameters serve as a rationale for setting the clinical objectives.[1,2,3]

The studies utilizing clinical samples from endodontic infections have potential to bring about colossal contribution to current data of pathogenesis of endodontic infections in health and disease. Also intervention strategies focusing on the microbial ecosystem and virulence have immense potential to serve as predictable therapeutic alternative.

Discussion

Endodontic microbiology

The untreated dental caries, trauma etc. may lead to endodontic infections like pulpitis, pulpal necrosis proceeding to apical periodontitis, apical abscess and cyst (Figure 1). The progress of infection into apical region can have serious bearing on the integrity of the tooth in the arch and systemic health of the individual. The failure of endodontic therapy is a concern not only for the patient but also for the clinician.

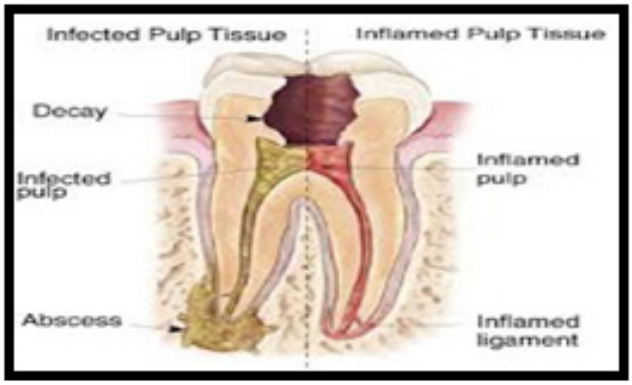

Figure 1 Pathway of Endodontic infection.

The heterogeneous microflora comprises of 65% bacteria, 30% fungi and 5% of other organism.[4,5] Lack of microbial specificity affecting the treatment outcome may be predisposed by the dearth of consistent evidence. The ability of the microorganisms to survive the most challenging environment of the root canal is remarkable. The complexity of root canal anatomy, nutrition supply, oxygen tension and microbial interaction makes the root canal environment very unique.[6] The advanced Molecular biology techniques are the forefront of microbial analysis. It provides rapid, sensitive and specific tools for the analysis of DNA, RNA and proteins.

Behavior of Microorganisms in Root canal

Most of the oral microorganisms have the inherent capacity to invade the pulpal space and deep into the dentinal tubules, isthmus, accessory and lateral canals (Figure 2). Studies have contradicted the development of apical periodontitis from necrotic pulp tissue and stagnant fluid in the absence of microorganisms. However, root canal flora comprises of more restricted species compared to oral flora, implying selective pressures playing role in the survival of few microorganism.

Taxonomy of root canal flora

The significance of identifying taxonomy of the root canal is to divulge bacterial combinations that might play key roles in the disease initiation, progression, resistance and treatment failures. The size and age of the inoculum, the degree of anaerobiosis, isolation technique and laboratory conditions influence the characteristics of the organism. There is a constant description of new organisms and taxa, resulting in current taxonomy of root canal flora (Table 1). Domination of facultative anaerobic bacteria makes up the significant part of the flora. Aerobic bacteria may be introduced later during the treatment. Hence the knowledge of the current taxonomy in particular geographical location is significant for interpretation of results.

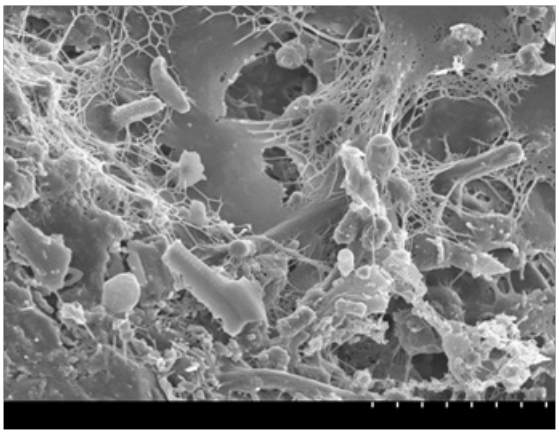

Figure 2 Bacterial aggregates in root canal.

Pathogenicity of endodontic microflora

The microflora of infected root canal has the ability to initiate periapical inflammation through combination of species. Prevotella and Porphyromonas resist abscess resolution by increasing the accumulation of leukocytes. In polymicrobial infections, phagocytosis, intracellular and growth factors might protect the host to certain extent. But in mixed cultures, obligate anaerobes can interfere with this defense mechanism of facultative anaerobic partners. Also synergistic lowering of local oxygen concentration by facultative anaerobic bacteria, facilitate the invasion and replication of anaerobic bacteria in polymicrobial infections. Pathogens produce resorption and tissue destruction enzymes and also inactivate human plasma proteins. The host defense mechanisms of immunoglobulins and complement factors, as well as plasma proteinase inhibitors and plasma proteins of the clotting, fibrinolytic and kinin systems play important roles in various phases of the microbial invasion. Data on microbial morphology provides clues for the identification of most microorganisms and physiological traits are often ambiguous.[7,8]

Molecular biology in endodontics

The current substantial knowledge of the endodontic ecosystem is by conventional culture technique. It may not provide an accurate data of the microbial load as many organisms fail to endure for identification under routine laboratory conditions.

The phenotypic identification have certain drawbacks namely impossible to culture large number of extant species, non recovery of all viable microbes, need immediate processing, expensive, time consuming (several days to weeks), low specificity, low sensitivity, microbiologist' expertise and need for transport media. They also not favor the culture of all species either due to their specific nutritional requirements and growth factors. The toxicity of the culture, injunctive substances released by other microbes or metabolic dependency can hamper the culture technique. All these factors "underestimated" the endodontic pathogens responsible for pulp and periapical infections. Hence the role of culture as a gold standard in microbial taxonomy is not completely justified.

This has made comprehensive data collection still unreality. This has paved way for culture independent techniques –Molecular biology techniques. The study of DNA, RNA and proteins has opened new avenues for clinical research that revolutionized the identification of new species, understanding micro flora and led to rapid diagnostic tool.[9,10,11]

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Table 1 Bacteria isolated frequently from root canals of teeth with pulpal and periapical disease

1***) Bacteroides melaninogenicus – Prevotella and Porphyromonas***
Prevotella species :
- Prevotella intermedia
- Prevotella nigrescens
- Prevotella tannerae
- Prevotella multissacharivorax
- Prevotella baroniae
- Prevotella denticola.

Porphyromonas species
- Porphyromonas endodontalis
- Porphyromonas gingivalis.

2) Tannerella forsythia

3) ***Dialister species***
- Dialister pneumosintes
- Dialister invisus.

4) Fusobacterium
- Fusobacterium nucleatum
- Fusobacterium periodonticum

5) Spirochetes
- Treponema denticola
- Treponema socranskii
- Treponema parvum
- Treponema maltophilum
- Treponema lecithinolyticum.

6) Gram-positive anaerobic rods
- Pseudoramibacter alactolyticus
- Filifactor alocis
- Actinomyces spp.
- Propionibacterium propionicum
- Olsenella spp.
- Slackia exigua
- Mogibacterium timidum and
- Eubacterium spp.

7) Gram-positive cocci
- Parvimonas micra
- Streptococcus spp. which include Streptococcus anginosus, Streptococcus mitisi, Streptococcus sanguinis and Enterococcus faecalis.

**Other bacterial detected more sporadically include:
- Campylobacter rectus
- Campylobacter gracilis
- Veillonella parvula
- Eikenella corrodens
- Neisseria mucosa
- Centipeda periodontii
- Gemella morbillorum
- Capnocytophaga gingivalis
- Corynebacterium matruchotii
- Bifidobacterium dentium
- Lactobacillus spp. like Lactobacillus lactis, Lactobacillus paracasei and Lactobacillus plantarum

The first Molecular Biology approach in Endodontics was made by Conrads et al.,[12] One of the earliest methods developed by Kary Mullis was Polymerase chain reaction (amplification). Isolation of previously unknown species from endodontic infection- *B. forsythus* and *T. denticola, Dialister pneumosintes* and *Filifactor alocis T. parvum* and *T. putidum* have been possible by PCR.[13,14] Many variants of PCR -Multiplex PCR, Nested PCR, Arbitary primed PCR, Quantitative PCR, Reverse Transcriptase PCR, Real Time PCR were eventually developed. Real time PCR identifies the target by fluorescently labeled probe. PCR products can be analyzed by Denaturing Gradient Gel Electrophoresis (DGGE).[15] Later with the introduction of checkerboard DNA hybridization, large number of DNA samples were hybridized from single /multiple samples forming a great tool in communal research (Figure 3).[16]

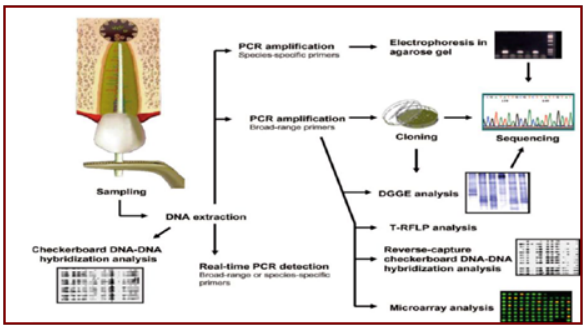

Figure 3 Various Molecular biology techniques and their stages of analysis.

Development of 16SrRNA gene sequencing has explored wide range bacterial phylogeny that has provided new lineage for the taxonomy. Novel gene and their products, new protein sequence, antimicrobial molecules, gene reassembly provides dramatic insight to the genomic function, structure heterogeneity of microbial organisms. The latest innovation in this regard is Metagenomics, wherein microbes are subjected to genomic analysis by cloning directly samples are studies directly from their environment. The libraries permit analyses of species diversity based on PCR independent approach and also comprehensive description of functionalities of the whole ecosystem. In this regard, Molecular Biology techniques (Table 2) provide invaluable information about the physiological and functional role of the oral microbiota, including species that have not yet been cultivated.[17,18]

Table 2 Molecular techniques and applications

Technique	Application
Polymerase chain reaction (PCR)	Single or multiple species identification
DGGE & T-FRLP	Analysis of microbial community structure by using amplified PCR products
Fluorescence in situ hybridization (FISH)	Abundance of particular species and their spatial distribution in tissues
DNA-DNA hybridization macro-arrays and microarrays, Specific PCR, nested PCR, multiplex PCR and real-time PCR	Survey a large number of clinical samples for the presence of target species-.
The 16S /18S rRNA gene sequencing	*Gene Targets for Microbial Identification*

Conclusion

The endodontic infections can be symptomatic and can have serious bearing on the integrity of the tooth in the arch. The presence of apical periodontitis is linked to systemic implications like-infective endocarditis, bacteremia etc. The role of microorganism in causing the root canal infections is well established. Contextually, community microbial profiling unveils the pathogens and serves as a rationale for setting the targeted clinical protocols. The dominance of certain species in some locations and geography-related pattern can been confirmed by the analyses. Also the correlation of various parameters from diagnosis to treatment outcome can provide valuable information. Although the incidence of virulence factors are more pronounced in hospital infections, endodontic isolates are exhibiting emergence of bacterial resistance to conventional regimens used in dental procedures. The different expression profiles of virulence factors can be explained by geographic differences, diet, infection stage and systemic condition.

The culture technique has been standard method of diagnostics in infectious diseases since many centuries. Due to time consumption, low sensitivity and specificity led to the era of molecular analysis. Genomic analysis is rapid, highly specific and sensitive and can directly analyze the sample. The major setback of the technique includes cost, overestimation of flora and inability to assess functional relationship. Hence the combination of both the techniques can expand and further refine the current knowledge regarding the species and communities associated with different clinical conditions. It can witness a huge change in the trend of treatment approaches.

Acknowledgments

None.

Conflicts of interest

None.

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