



EXPLORING BACTERIAL DIVERSITY OF ENDODONTIC MICROBIOTA BY CLONING AND SEQUENCING 16S rRNA

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Abstract

This particular study emphasizes the scientific methods that can be utilized to explore the diversification of Endodontics Microbiota by the cloning and sequencing of 16S rRNA. With this study, an overview of the diverse bacterial infection that can affect the root canal of the oral cavity has been demonstrated. A comprehensive view of the infection trends has been determined. Further, the role of 16S rRNA that helps in conducting the cloning and sequencing process has been discussed in this study.

Keywords: Endodontics Microbiota, cloning, sequencing, bacterial infection, rRNA, endodontic infection, pathogenesis.

Introduction

The practise of root canal therapy over the years has come up with innovative ideas to treat the infected areas effectively. Understanding microbiology and evaluating the endodontics pathology it is very essential for the world to gain knowledge about the conventional treatments that are associated with it. While many studies are aiming to revolve around the innovation of resistant organisms that will eliminate the sources of the infection, this study will emphasize on the idea of exploring bacterial diversity of endodontic microbiota that is analysed by cloning and sequencing 16S rRNA.

This study will therefore, highlight the concept of endodontic treatment and its significance to facilitate the modern world with immense scopes. Since cloning and sequencing 16S rRNA is an essential topic that has gained limelight over the past years, the causes and the functional features of the root canal therapy will be gained. Based on the importance of cloning and sequencing the bacterial diversity and the kinds of infection affecting the dental surface will be gained. Since the purpose of the study is based on the evaluation of bacterial diversity concerning the endodontic infections, the cloning and sequencing 16S rRNA will be analysed through this study.

Concept of endodontic treatment

Nowadays with the evolution of the modern world, the process of endodontic therapies has enhanced prevention of teeth. With the increased success rate of the endodontic procedures, the therapists are capable of combating the infection by curing the system effectively (Nóbrega et al., 2016). Especially for endodontic infection, the curing stages are very less as several chemical and physical factors are there that actively participating to create inflammation around the dental system. The main function of endodontic treatment is to eradicate the infection and prevent the infective stages of the perradicular tissues. Therefore, to understand the functional traits of endodontic microbiota it is very essential to evaluate the success rate that helps in the prevention of reinfection. Reaching the pulp of the teeth is a prime factor that affects the dentinal tubules causing lesions. Apart from that, the endodontic infection can occur within the mouth cavity including the Pathways of bloodstream periodontal membrane faulty Restoration and many more through other ways.

- Dentinal tubules- Following the dental procedures the microorganisms can enter the pulp following a centripetal direction (Nóbrega et al., 2016). With this, the bacteria gain access to the distance between the border of the Carriers lesson and the pearl that is not less than 0.3 mm.
- Open cavity-With the direct pulp exposure to the infection the bacteria can attack the open cavity by breaking the physical barriers and imposing the dental structures by leaving a sceptical contact of the oral and the environment.
- Bloodstream- transient layer of bacteria can occur within the healthy individual where the bacteria is present in the bloodstream and causes inflammation to enhance the endodontic infection.

- Faulty restoration- Occlusion infection can also occur through salivary contamination where the bacteria can gain access to the periapical tissue (Attavar and Hegde, 2018). This is very difficult to restore as the lateral Canal migrating from the infected tooth to healthy pulp can cause the spreading.

Apart from that, several studies have shown more than 800 bacterial species are found within the oral cavity that harbours several infections and progress in causing endodontic infections. Once the root canal is affected the infection progresses to infect the apical portions where the tissues develop periodontitis. Since endodontic infections are polymicrobial in nature, with the presence of obligate anaerobic bacteria's it is possible to infect the surroundings of the mouth cavity extensively.

Further, if samples are collected and cloned with the help of cloning and sequencing 16S rRNA, it is possible to reduce the infections and gain the knowledge of bacterial diversity (Xiao et al., 2016). Considering the bacterial domination there are various microorganisms that affect the intraradicular and extra radicular parts of the mouth cavity and persist the infection for the long term. For intraradicular effects, the endodontic pathogens that cause the infection include saccharolytic species like *Prevotella* and asaccharolytic species *Porphyromonas*.

Prevotella species detected in the endodontic infections include *Prevotella intermedia*, *Prevotella tanneriae*, *Prevotella denticola*, *Prevotella baroniae* whereas the *Porphyromonas* species includes *Porphyromonas endodontalis* and *Porphyromonas gingivalis*. Various types of gram-positive cocci are present within the endodontic infections that include *Streptococcus anginosus*, *Streptococcus mitisi*, *Streptococcus sanguinis* and others (Rodrigues et al., 2019). Few phylotypes are there that are recognized to play a key role in generating the pathogenesis of the periodontitis including cloned eubacterium, olsenella, cytophaga.

In the case of purulent inflammation, the extraradicular infection causes a huge effect on the functional process of the root canal treatment. *Actinomyces* sp, *Treponema* sp are the main bacterial cause of ocular infection considering the extraradicular sources.

In order to understand the functional attributes of cloning and sequencing, it is very important to get the idea of DNA and its properties. Cloning actively participates in inserting an arbitrary DNA fragment that is having similar primer sequences in order to gain functional advantages. Before undertaking the advantages of Sequencing, it is very essential to clone that helps in improving the quality and the length of the sequence thread (Slaton et al., 2017). The 16s ribosomal RNA codes for the relevant RNA component that helps in distinguishing the bacterial sequence in order to enhance the treatment purposes. 16S rRNA gene sequencing

is particularly used to shorten the time in order to identify the slow-growing bacteria in the clinical diagnosis system. This activity participates in general anti antibiotic treatments that help in hearing the endodontic diseases effectively.

Further culture medium, species identification and phenotype tests are the main functions of the RNA gene that helps in introducing alternative gene targets to reduce the functional abnormalities extensively (Zhang et al., 2020). With the proper analysis of nucleotide variation in rRNA operons acts as a single genome that it helps in accurate identification of the bacterial isolates. In addition, since this study is aiming to focus on the evaluation of various bacterial diversity in endodontic infections, cloning and sequencing of 16S rRNA will help in gaining accurate ideas of the study diversity extensively.

Diversity of endodontic microbiota

All the viruses, archaea and fungi contribute to the microbial diversities in causing endodontic infections; bacteria's are the most featured microorganisms that help in occurring this kind of infection predominantly. The report says that more than 500 unique bacterial taxa belonging to various genres and phyla have been identified causing endodontic infections (Yousuf and Mishra, 2019). With the abundant occurrence of firmicutes, bacteroidetes, actinobacteria, proteobacteria there are several cultivated phylotypes that enhances the infection within the cavity.

Fungi types are associated with the *Candida sp*, *Candida albicans* that causes endodontic infections. With other sources of archaea and virus, infections are traditionally recognized through the diverse group of prokaryotes. The archaea are normally recognized as extremophiles that are non-extreme to the environment. Apart from that, periodontal diseases that cause chronic apical infection detect methanogenic archaea. With the protein coat viruses, they require a host cell in order to infect and replicate (Siqueira and Rôças, 2017). Thus, it can be easily stated that the viruses cannot survive effectively in the necrotic root canal.

In the case of few non-inflamed vital pulps, the occurrence of viruses has been observed that causes pathogenesis in the roots of the oral cavity. Since the types of infections within the mouth cavity are of two types, with intraradicular microorganism, the root canals are highly restricted to cause defence barriers. With the studies, *Enterococcus faecalis* is identified as the most common species that are recovered from the root canals of the dental sets. If the endodontic therapy fails there are various kinds of infections occurring within the canals that can cause a huge impact concerning the survival chances of the root canal (Verma et al., 2018).

The success rate of persisting the infection is dependent on the functional aspects of microorganisms. This includes-

- Surviving in the poor nutrient environment
- Can survive in the presence of medications (sodium and calcium hypochlorite)
- Can survive in extreme environments like high temperature, low PH, high salinity.
- Form biofilms and sustains through medicated processes
- Can endure the prolonged period of starvation

Several microorganisms are found in endodontic infections that commences the growth of oral cavity. It gains the entry from the pulp tissue of the root canals and enhances virulence factors that play an effective role in the endodontic infections. Few virulence factors that help in evaluating the endodontic infections are discussed as follows-

- LPS- It is also known as endotoxin that plays an integral part in creating the cell wall of the gram-negative bacteria's (Paulino, 2017). Lipopolysaccharides cause the mobilization of immunosurveillance mechanism within the pulp that destructs the periapical bones.
- PG- Peptidoglycan layers are the major components that help in generating Gram-Positive cell walls of bacteria. In the case of lice, peptidoglycan is released that helps in enhancing the immune system to proliferate the boosted presence of LPS.
- LTA- LTA is a cell wall component that is formed of echoic acid and lipids. LTA or lipoteichoic acid share several pathogenic properties with LPS that interact with the circulating anti by bodies to activate the cause of damages.
- Fimbriae- These are the filamentous macromolecules that are found in the Gram-Negative bacteria's, which are involved with an attachment of surface creating interactions with the bacteria (Burczynska et al., 2017).
- Capsules- capsules are the organised layer of cell walls that are generally composed of polysaccharides and utilize to inhibit the complement activation of phagocytes.

Apart from that, several other virulence factors are there like extracellular vesicles, exotoxins, short-chain fatty acid, polyamines and many others that enhance the occurrence of bacteria within the orthodontic parts and actively participates in providing clinical signals to enhance root canal infections.

Microorganisms found in successful endodontic treatments enter into the root canal from leakage. It makes it very difficult for the experts to differentiate between the primary and secondary infection as the primary infection is viable that causes difficulty to mitigate. The main objective of endodontic therapy is to make the root canal acceptable to sustain environmental changes (Zhou et al., 2017). With *Enterococcus faecalis* considered as the essential factor that is considered as the main reason to cause infection, it is very essential to analyse the pulp problems and many more. The occurrence of persistent lesions, therefore, can enhance the infection that must be reduced in order to enrich the immune system of the patients.

Exploring bacterial diversity of Endodontic Microbiota by cloning and sequencing 16s rRNA

The main characterization of microbial communities revolves around infecting the endodontics system that helps in creating a prognosis to understand the district strategy of the treatment. Sainik gas 7 microorganisms that affect the endodontic system the purpose of the study will provide in-depth knowledge of the bacterial diversity that can be analysed with the help of 16S rRNA gene. For this, the researchers must actively participate in collecting samples of the root canal that exhibit lesions of periapical infections (Tawfik et al., 2018). 16S rRNA bacterial genomic libraries must be constructed cloned and sequence that will help in estimating the bacterial diversity.

Acute endodontic infection I most commonly treated procedures which if left untreated can result in life-threatening conditions. With pain palpitation, the apical connective tissues cause intense inflammation that enhances the complications within the root canal system. Microbiota that is associated with endodontic infections are heterogeneous in nature and can actively participate in curing root canal infections. Since more than a thousand bacterial species belonging to 15 phylotypes are identified as oral cavities, the diverse expansion of the isolated endodontic infections based on RNA sequencing can act as an important tool in identifying and cultivating the pathogen (Widmer et al., 2018). Direct amplification of the 16S rRNA gene followed by the stages of cloning and sequencing will help in evaluating the bacterial cultures of the human oral cavity system. Proper evaluation of oral microbiome of the human race utilizing the advanced techniques of metagenomic approaches will result in the identification of the 16S rRNA gene sequence analysis to enhance the clonal procedures.

Sample selection followed by the analysis of the dental records is very essential to evaluate. Considering the root canal infections, one can easily gain the knowledge of periapical lesions, pulp necrosis and other

periodontal diseases that can function to heighten the restoration processes. With the application 16S rRNA sequencing, the root canal samples can be extracted considering aseptic techniques (Byrne et al., 2018). Following the sampling processes like dental caries and existing restorations, And isolation can function well to conduct the procedure efficiently. Isolating the tooth sample and progressing with disinfectant procedures, the entire procedure of sampling can be performed. Considering a full-length process of moistening and sterling the canal can be transferred into a test tube that will include the presence of viability medium.

For the sequencing process, the 16S rRNA gene products must be ligated to progress with the cloning medium. With the application of PCR (Polymerase Chain Reaction) technique, more than 500 bases of sequences will be gained. Slow assessment and gaining ideas of the polymicrobial infection the bacterial viability will be evaluated. Apart from that from dead cells in endodontic infections, the possibilities to detect DNA is very low (Lang et al., 2017). The primers designed to generate large numbers of DNA detection reduce the functional capabilities by enhancing the identification of root canal infections. Since the presence of Gram-Negative Bacteria is essential, it helps in gaining an idea of the inflammatory and clinical significance. Therefore considering the clonal analysis of primary infections a wide diversity of species can be identified that are responsible for the root canal infections. Gaining an idea of the variety of bacterial communities, the role of pathogens in heightening sustainability must be analysed. In addition to that, analysing the clones the sequences can be depicted well that will further participate to provide in-depth knowledge of the prevalent taxa of the phylogenetic groups.

Since root canal therapy or endodontic treatment helps in curing the cavities in the teeth, the identification of diverse bacterial infection will actively participate in preventing the malfunctioning (Soares and Faveri, 2019). In addition to that, it is the highly specialised segment of dentistry that provides a purpose to ensure that the diagnosis and the treatment are done in an effective way. People across the global arena have a vague belief that the process of endodontics is very painful while with the correct measures undertaken by the population, it is very essential to gain knowledge of the commensal microbiota system that helps in gaining an idea of the other factors, which can regulate the infection. Pathogenicity in the human body is caused by the infection of several microorganisms.

This includes parasites, protozoa, bacteria and other pathogens that adheres, colonizes, survives and propagates to elevate the infection. Availability of nutrients and other factors can actively participate in increasing the colonizing modes (Zhao et al., 2017). Further, oral microbiota progresses the infection that alters the nutritional

status by encouraging the proliferation of the system. With this, the diverse activities of the bacteria's are explored and the functional approaches of the 16S rRNA are gained. Since infection in root canals does not occur randomly but is associated with the amalgamation of microbial factors that infects the ocular cavity, it is very essential to treat, the infected parts properly without which it is not possible to sustain.

Significance of the study

The study is very significant as it provides an in-depth knowledge of the bacterial communities that are associated with the endodontics infections along with the importance of 16S rRNA. With the treatment of infected pulp, the elimination of infection sustaining the protection from decontaminated tooth helps in keeping the oral cavity healthy (Kugaji et al., 2017). With the removal of pulp followed by the cleaning measures, the treatment will help the patients from any further infection and irritation. Understanding the proper use of cloning and sequencing in order to reduce the pain, the act will help the patients to sustain an effective life. Gaining ideas of the bacterial diversity that are associated with the 16S rRNA sequencing, the functional values can be gained. Further, considering the proper treatment of the endodontics, it is possible for the root canal treatment to involve the shaping of the entire procedure that will assist the patients with utmost relief (Gao et al., 2016). With the help of this study, the readers will be able to gain the ideas of the importance of clones that helps in identifying the bacterial family classes. Constructing the genomic libraries, the concept of novel phylotypes with the basic knowledge of the endodontic samples will be gained. Considering the strategies that help in identifying the infected endodontic system it is very essential to analyse the trends of the bacterial proliferation that helps in the functional aspects. Therefore, this study will actively participate to not only provide knowledge in evaluating the diverse range of bacteria's but to also gain facts of the proper 16S rRNA sequencing and cloning (Wang et al., 2016). With the establishment of correct prognosis and distinct strategies, bacterial diversity and infections will be identified extensively.

Conclusion

Endodontics is a therapy that helps in curing the dental diseases affected within the oral cavity. Many perceive that the root canal therapy is very painful as it involves hard equipment assistance whereas based on the functional advantages it can be clearly stated that with endodontics the dental canal necrosis can be easily lowered that will help in providing long term relief to the patients. In addition to that, the endodontic treatment enhances the patient's immune system that prevents the occurrence of any kind of infection effectively. Based

on the microbial attacks and the sustainable microorganisms, the diverse nature of the bacteria's can be gained. This study has therefore attempted to highlight the diverse infection of endodontics bacteria's along with their functional values. Gaining knowledge of the endodontic treatments the readers will be able to explore the bacterial diversity of Endodontic Microbiota based on the 16S rRNA cloning and sequencing.

Considering the future research, it is very essential to access the scopes that will help the aspiring researchers in conducting future experiments. With this study, the researchers will be able to access the samples that will assist in treating the experiments with the use of advanced PCR technologies.

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