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PCR-Based Detection of Red Complex Bacteria: Techniques, Applications, and Clinical Insights

Avani Tomar¹, Priyanka Patel^{2*}, Preeti Kumari³, Garima Sharma⁴, Salil Singh⁵

¹ Dissertation trainee, Department of Zoology and Biotechnology, Vikram University, Ujjain

² Director, Rapture Biotech, Ahmedabad, Gujarat, India

³ Research Associate, Research Department, Rapture Biotech, Ahmedabad, Gujarat, India

⁴ Assistant Professor, Department of Zoology and Biotechnology, Vikram University, Ujjain

⁵ Assistant Professor & Head, Department of Zoology and Biotechnology, Vikram University, Ujjain, Madhya Pradesh, India.

*Corresponding author's email: gujarat.rapturebio@gmail.com

Abstract

Periodontitis is a Chronic inflammatory infection which is most frequently caused by activation of dysbiotic groups of microbes, which in the majority of the cases consist of *Treponema Denticola*, *Porphyromonas gingivalis* and *Tannerella forsythia*, which both are referred to as red complex. The complication of Periodontitis can cause loss of periodontal and alveolar bone, which means that there is tooth loss and oral complications. besides that, it can cause complications in CVS, Diabetes Mellitus, or Alzheimer's. Normal methods like bacterial cultures cannot identify these pathogens. Revolutionized PCR is really getting better in the detection of the pathogen. Timely detection is important for better prognosis. In this study, we will talk about the systematic influence and the effects of red complex on the oral habitat, and in addition to that, we will talk about the modern diagnostic methods used for its detection.

Keywords

Periodontitis, Red Complex, *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, PCR (Polymerase Chain Reaction), Virulence Factors, Systemic Inflammation, Endodontic Pathogens.

1. Introduction

Oral flora have the microbes that have the ability to infect the human being's oral cavity. Food travels through the mouth, where it is chewed and combined with saliva en route to the

stomach and intestines. The human mouth contains numerous different environments: teeth, the area between gums and teeth (gingival sulcus), the tongue, cheeks, the hard and soft palate, and tonsils [1]. Periodontal disease is an infectious disease, and the combination of microbes involved is very complex. Research has shown a clear link between periodontitis and a small number of microbial species. They are *Porphyromonas gingivalis*, *Tannerella forsythia*, *Aggregatibacter Actinomycetemcomitans*, *Treponema Denticola*, and *Prevotella intermedia*. The surface of the tongue is filled with pathogen. Their metabolic byproducts are also thought to be responsible for halitosis. Being able to identify and quantify these pathogens in clinical samples would be a tremendous help in diagnosing and treating periodontitis and in understanding how halitosis happens [3]. Several quantitative analysis methods are employed to detect oral pathogens. Flow cytometry, DNA-DNA hybridization, and real-time PCR are among them. These instruments may assist us in learning about the role of such pathogens in oral health and disease [5]. As we are aware that three specific bacteria ie *T. denticola*, *P. gingivalis*, and *T. forsythia*—tend to roll together. Also termed as the “red complex”, and they’ve been heavily linked to how chronic periodontitis gets worse over time. A recent study looked into what kind of bacteria is in the deep pockets of gum tissue in patients with chronic periodontitis. *P. gingivalis*, *T. denticola*, and *T. forsythia* showing up most of the time. But *P. gingivalis* or *T. denticola* almost never showed up without *T. forsythia* being there too. Mineoka and their team backed this up in another study—they also noticed that *P. gingivalis* and *T. forsythia* tend to co-exist. What’s even more wild is that *T. forsythia* is considered more common than *P. gingivalis*. That kind of hints at the idea that *T. forsythia* might be the one that shows up first before *P. gingivalis* and *T. denticola* move in. *Porphyromonas gingivalis* members of the *P. gingivalis* are gram negative. They are anaerobic and are the size of apx. 0.5 0.8 to 1.0 3.5 μ m diameter. The colonies are often whitish coloured when we grow them on the blood agar surface. With the passage of time, the colonies turn into the dark edged towards their center with deep red colour. Which looks just like proto heme [7]. *Tannerella forsythia* Spirochetes are long and thin. They look like corkscrew and are gram negative anerobic bacteria whose mortality can be seen readily by phase contrast under microscopic examination. The spiral-shaped cells of *T. denticola* are covered. They are enveloped with a fragile envelope. They produce 4 flagella which are intertwined with cytoplasmic cylinder. The predominant outer sheath is called major outer sheath protein (MSP). *Treponema denticola* is from the group called Cytophaga-Bacteroides family. It is anaerobic and gram negative. *T forsythia* is linked with periodontal disease. This includes gingivitis. The infection is more common in unhealthy overweight

women. The condition is thought to be the primary cause of adult tooth loss, and it can lead to both psychological repercussions like social disengagement and low self-esteem as well as functional impairments like trouble speaking and chewing [7].

2. Pathophysiology of Red complex bacteria

2.1. Clinical presentations

Periodontal diseases, is a global health issue due to their chronic nature and its etiology. These are rather the inflammatory conditions, resulting from intricate interactions between the microbial biofilms and also the available host immune response that mostly lead to eventual tissue destructive processes and loss of alveolar bone. If we do not treat them properly, they result in the irreversible damage to the mouth. But also cause issue in self-esteem and aesthetics of the body. Aside from its local effects, periodontitis has been linked to systemic health effects, with growing numbers of the links which suppose they are associated with other bodily issues. Periodontitis doesn't only effect your mouth, but also other disease. like heart disease, diabetes, pregnancy complications, and even brain disorders like Alzheimer's. Studies suggest this happens because the infection can keep coming back, bacteria can enter the bloodstream, and the body's immune response can get thrown off. All of that can make other health issues worse. As a result, periodontitis is now known not only as an oral health issue but as a major risk factor for broader medical complications, underscoring the importance of early diagnosis, effective intervention, and interdisciplinary management strategies [6].

2.2 Virulence Factors

The red complex, *P. gingivalis*, *T. denticola*, and *T. forsythia* presents the climax community in the biofilms. When we do not have the orange complex, we don't really see the red complex. The red complex is strongly linked with the bleeding and pocket depths on probe. For example, with the increase in the number of pocket depth we have the increase in the number of *T. forsythia* and *P. gingivalis* [5].

2.3 Role in Systemic Diseases

The role of the pathogens of the red complex goes beyond the oral cavity itself. Studies say that *P. gingivalis* and its factors can enter the circulatory system and cause cardiovascular diseases by inducing endothelial dysfunction and atherosclerosis. Insulin resistance is also seen in the periodontitis-linked inflammations and thus in aggravating diabetes mellitus.

There have been recent discoveries indicating a close link between *P. gingivalis* and Alzheimer's disease as its toxins have also been found in the brains of affected patients, which can lead to neuroinflammation and cognitive impairment.

3. Diagnosis and Detection

3.1 Traditional Methods

Different diagnostic procedures have been utilized to detect the Red Complex bacteria. Culture methods are anaerobic but tedious and possess low sensitivity. Immunofluorescence and DNA probes enhance specificity but cannot be used to detect all bacterial species, though Microscopy, Biochemical test, Sublingual plaque sampling are also some of the methods previously used for the bacterial identification and therefore molecular methods are more consistent for early detection. Periodontal pathogens like the red complex bacteria *P. gingivalis*, *T. forsythia*, and *T. denticola* have for a long time been microscopically analyzed as foundational techniques in microbiology. Typically, the first step gram staining reveals these organisms to be gram negative owing to their thin and pink peptidoglycan layer. While some classification is given by Gram staining, it does not provide specificity at the level of species. For the case of *T. denticola*, dark field microscopy has particular relevance as this organism is a spirochete which has highly noticeable spiral form as well as motility both of which can be seen through a microscope in an unstained fresh sample. Researchers can use this method to observe bacteria in motion, and enables them to evaluate passive motility which is frequently associated with pathogenicity [17]. The battlement steps utilize specialized media devoid of oxygen to culture bacteria, which is critical since red complex bacteria are strict anaerobes. This technique enables the selective isolation of organisms and can be useful for other processes like antibiotic testing and virulence evaluations. *T. forsythia* and *P. gingivalis* usually grow on enriched media containing hemin and vitamin K. However, these approaches have serious drawbacks, especially with *T. denticola*, which has an intricate set of nutritional needs along with being slow-growing and tough to culture. Culture work, while difficult and time intensive, remains unparalleled for confirming pathogen existence and studying their biology in structured settings [18]. The initial step in most of the periodontal microbiological investigation is Subgingival plaque. Samples are collected using sterile paper point or even curettes from periodontal pockets directly. This is minimally invasive procedure; which provides a targeted approach to isolate bacteria from specific disease sites allowing Clinicians or researchers to correlate microbiological findings with

clinical symptoms. The analysis of collected plaque can be done by using Microscopy, Culture, Biochemical tests or modern molecular technique. This method is precise and its ability to reflect the microbiological composition of the niche most associated with periodontal disease progression [19][20].

3.2 Emerging Molecular Diagnostic Tool

Polymerase chain reaction (PCR) is popular because of its specificity. A PCR can identify even low bacterial loads that may go undetected by routine methods. The accuracy makes it preferred by the researchers, cutting down the time taken for bacterial identification considerably. It has the added benefit of identifying more than one pathogen at a time, enabling detection of *P. gingivalis*, *T. forsythia*, and *T. denticola* in patient samples, and this is significant for directed treatment planning [21]. Polymerase Chain Reaction (PCR), is a molecular technique. It allows for selective DNA sequence amplification of specific bacteria using primers. In periodontal diagnosis, PCR aims at genes such as 16S rRNA because it has conserved and species-specific regions that will render it effective for the identification of red complex bacteria *P. gingivalis*, *T. denticola*, and *T. forsythia*. Its sensitivity and specificity make PCR an effective assay for the detection of bacterial DNA in oral samples, including saliva or subgingival plaque. Standard PCR assays are improved by real-time monitoring of amplification by fluorescent indicators of DNA in Real-time PCR (qPCR) Greater fluorescence indicates greater amounts of DNA. This allows for the quantitation of bacterial load which is critical for determining the level of the infection and also for the development of periodontal therapy. With the use of a series of primers for various pathogens in a single reaction, Multiplex PCR allows for the detection of several species of bacteria simultaneously. Now this process less time-consuming, cost-effective, and requires lower sample volume, which is advantageous for limited specimens such as subgingival plaque. It is used extensively in studies as well as in clinical practice so that a general understanding of oral flora and their role in periodontal diseases could be attained [20][21].

4. Future Prospects

Research has increasingly shown that there is a definitive connection between periodontitis and systemic diseases with more emphasis on early detection of the red complex and its treatment. Increasing evidence have suggested that periodontal pathogens, mostly *P. gingivalis*, have the capability to move from the oral cavity into the systematic circulation

causing systemic inflammation. Interestingly, *P. gingivalis* and its virulence factors, i.e., gingipains, have been isolated from the brain tissue of patients with Alzheimer's disease, suggesting that there is likely to have link between chronic periodontal infection and neurodegenerative pathologies. Due to the multifactorial etiology of periodontitis, research continues to understand its causative agents and establish new therapeutic strategies. Since we have so many components to look after such as microbial biofilm, host immune system, host genetic susceptibility, and environmental factors has made the disease process complex. Hence, it is necessary for us to detect the causative agent early on by using better methods i.e., molecular detection assays like polymerase chain reaction (PCR), because the come in handy when we need early detection and specific treatment for specific pathogen [7].

5. Newer Diagnostic Tools

PCR has transformed microbial diagnostics because of its high accuracy, specificity, and sensitivity. As opposed to conventional culture methods, PCR allows the identification of uncultivable and phenotypically variant bacteria much more rapidly and accurately. It offers better differentiation of periradicular disease-related microbial populations. PCR-based investigations have been able to identify erstwhile unknown root canal pathogens such as *T. forsythensis*, *T. denticola*, *Dialister pneumosintes*, and *Prevotella tanneriae* at high frequencies. Fastidious bacteria like *P. endodontalis*, *P. gingivalis*, and *Eubacterium spp.* have also been found in considerably larger numbers compared to those identified using culture-based systems. The application of PCR amplification, cloning, and sequencing has greatly broadened our knowledge of endodontic microbiota and its implication in disease pathogenesis [10]. *P. gingivalis* is the main pathogen when it comes to gum disease. It's commonly found in the subgingival plaque of patients dealing with periodontitis, but its less common in healthy adults or children [11]. Now, since saliva plays a big role in spreading bacteria around, checking if *P. gingivalis* is in the saliva can actually tell us a lot about how it might be getting passed from one person to another. The problem is, traditional culturing methods don't really catch it. Plus, some culture media contain vancomycin, which blocks its growth. That's where PCR comes in handy. It's super sensitive and specific, and it can pick up *P. gingivalis* DNA even when there's a mix of tons of other bacteria. But PCR results can sometimes get obstructed by PCR inhibitors which can lead to false negatives. Even though using PCR to detect *P. gingivalis* from plaque is pretty standard by now, its use with saliva samples is still something that needs more research [12]. *T. denticola* is very difficult to

culture because it has strict anaerobic growth requirements. Most of the Conventional culture-based approaches have often failed to detect spirochetes, even though they play an important role in endodontic infections. According to research, almost 90% of endodontic infections include at least one species of spirochetes. Real-time PCR is a breakthrough method for identifying *T. denticola* without the requirement of anaerobic culturing conditions. Real-time PCR is rapid, sensitive, and quantitative detection, and it gives novel information about the role of *T. denticola* in peri radicular diseases [13]. *T. forsythia* identification uses a real-time PCR (qPCR) and involves DNA extraction from oral samples like subgingival plaque or saliva, followed by amplification of species-specific genes such as the 16S rRNA or tfsA/B using specifically designed primers and a fluorescent probe. As that, the DNA is amplified, fluorescence is increased, allowing the real-time PCR machine to quantify and measure the bacterial DNA. The earlier the fluorescence is observed (low Ct value), the higher the bacterial load. This method is very sensitive, specific, and especially appropriate for the detection and follow-up of *T. forsythia*-induced periodontal infections.[21]

6. Conclusion

If the patient is not treated on time, the pathogen can cause progress its issues and ultimately cause the loss of the tooth. These relations suggest that the red complex bacteria is the secondary reason for the issues that happens in the body. Traditional culture-based, diagnostic methods for periodontal pathogen identification continue to be hindered by their relative low speed, specificity, and sensitivity. The condition is thought to be the primary cause of adult tooth loss, and it can lead to both psychological repercussions like social disengagement and low self-esteem as well as functional impairments; setting the stage for point-of-care diagnostic device development capable of eventually achieving early detection and prompt therapeutic treatment. Finally, integration of novel technologies into evidence-based care will potentially enhance early detection, encourage better patient outcomes, and decrease the aggregate public health burden of periodontal disease.

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8. Reference

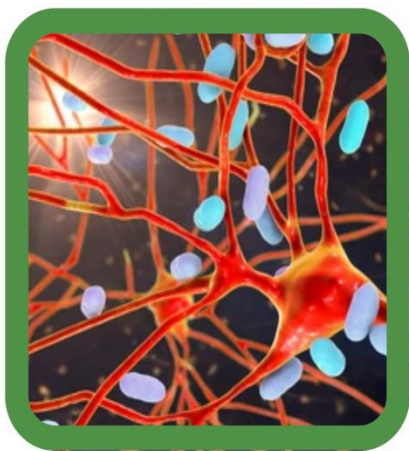
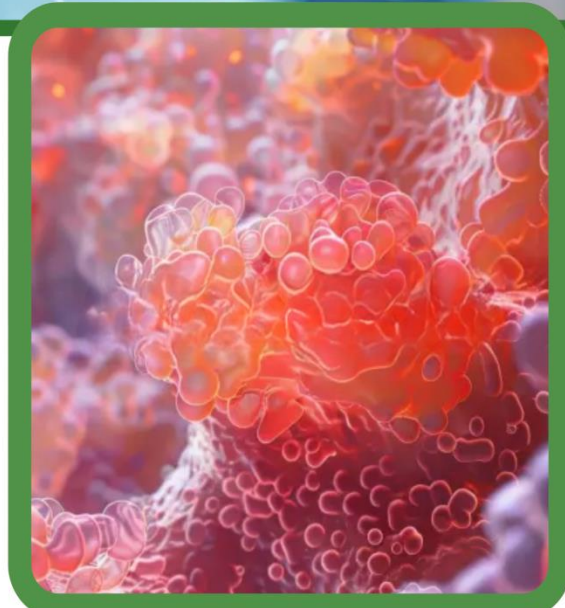
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