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Molecular Study of *Porphyromonas Gingivalis* Strains With fimA Genotypes in Periodontitis Patients

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ABSTRACT

Background and Aim: *Porphyromonas gingivalis* belongs to the phylum Bacteroidota which are non-motile, Gram-negative, rod-shaped, anaerobic, and pathogenic bacteria. It is found in the oral cavities where it is implicated in periodontal disease. This study aimed to isolate and identify *Porphyromonas gingivalis* from the oral cavities of patients with gingivitis and periodontal disease in Misan Governorate Center, Iraq.

Materials and Methods: The samples were collected from 50 patients aged 11-70 years old, including 21 (43%) females and 29 (57%) males. The Gram-negative pathogenic bacterial isolates were found to be the most frequent bacterial population. After incubation of bacteria on blood agar, the plates were examined for small, shiny, coccobacilli, black-pigmented, and mucoid colonies. We also tested the medical sensitivity of some pathogenic bacterial strains against antibiotics such as Cefoxitin, Doxycycline, Ciprofloxacin, and Nalidixic acid.

Results: The strains showed 100% resistance to the drugs used in the experiment. Periodontitis is caused by heterogeneous endodontic bacteria, which can be identified using species-specific PCR assays. The study successfully identified 100 anaerobic bacteria isolates using Vitek2 and 16S rDNA gene sequencing. The *Porphyromonas gingivalis* targeted region was found to be related to its ability.

Conclusion: Identifying and quantifying *Porphyromonas gingivalis* and other periodontal infections using various methods in plaque samples is crucial. *Porphyromonas gingivalis* has filamentous components on its cell surface called fimbriae (FimA), which are believed to be crucial for the resistance, colonization, and invasion of the periodontal tissues. The most important cause of gingivitis was found to be *Porphyromonas gingivalis* bacterial isolation and the age group from 31-70 or more were the most affected by gingivitis and periodontitis. According to the molecular analysis, it was also discovered that the genes of Fimbriae are among the most significant factors that promote the disease of gingivitis and periodontitis.

Keywords: Bacteria, Fimbriae, Gingivitis, Periodontitis, Polymerase Chain Reaction, *Porphyromonas gingivalis*

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1. Introduction

It is thought that at least 700 different bacterial species exist. The gingival sulcus is the area of the oral mucosa that has been the subject of most research in this field. The gingival sulcus, which lies between the gingiva and the tooth's hard surface, is home to the microbial populations that communicate with mucosal epithelial cells **(1)**. Dental biofilms are formed by interacting bacteria with this area and the formation of layers on the living and nonliving surfaces. These communities work together to protect tooth surfaces from non-oral microbial invasion and balance the acid and alkali production **(2)**. They can inhibit the growth of germs linked to the disease and can be influenced by the surface charge, surface

energy, roughness, and topography. Biofilms also contribute to tooth decay and shorten the lifespan of dental prostheses and restoratives **(3, 4)**. They have communication capabilities and are difficult targets for the immune system and medications due to their durability **(5)**. *Porphyromonas gingivalis* is a Gram-negative, anaerobic, non-motile, and non-spore-forming bacterium **(6)** that can cause local infection in periodontal tissue surrounding implants, causing inflammation and systemic disease through bacteremia, persistent inflammatory cascade activation, toxins dissemination, and pathogen trafficking **(7)**. *Porphyromonas gingivalis*, a keystone pathogen linked to periodontitis and Alzheimer's disease, lives in the root surfaces, gingival crevicular fluid, and gingival epithelial cells. It is found in patients with or without periodontal disease in the tonsil region, tongue, and buccal mucosa **(8)**. It is asaccharolytic and requires iron for survival. *P. gingivalis* is a member of the red complex of periodontitis-related anaerobes, producing black-pigmented colonies after 3-7 days of incubation **(9)**. *P. gingivalis* plasma membrane is crucial for oral pathogen growth and survival **(10)**. It contains virulence factors like outer membrane vesicles, lipopolysaccharides, gingipains, hemolysin, and hemagglutinins. Most strains have small filamentous structures called fimbriae that proliferate outside the outer membrane, facilitating biofilm formation and bacterial adherence to the host cells **(11-13)**. Fimbriae rely on the factors like staterin, fibrinogen, fibronectin, lactoferrin, and proline-rich proteins **(11, 14)**. They allow *P. gingivalis* to adhere to the host tissues, interact with other oral bacteria, and form biofilms. They also bind to TLR2 and promote inflammatory responses such as bone resorption **(15)**.

This pathogen expresses two distinct fimbria-molecules on its cell surface, one of which is composed of a subunit protein (named FimA or fimbrillin) encoded by the fimA gene, and termed long or long fimbriae, while the other consists of a subunit Mfa protein encoded by the mfa1 gene and termed short, minor, or Mfa fimbriae (henceforth referred to as simply long and short fimbriae)

The purpose of this study was to identify the patients with chronic gingivitis who carry the *P. gingivalis* fimA type I and type II genotypes.

2. Materials and Methods

Sample Collection

Fifty patients aged 11 to 70 years old who were visiting various dental centers in the heart of Al-Amarah City provided samples for periodontitis and gingival disorders, beginning on 21 November and ending on 6 February 2023. The samples from oral cavities included those with periodontitis and gingivitis of 5 mm depth of periodontal pocket.

Culture of Sample

Gingival Crevicular Fluid (GCF) samples collected from the patients were inoculated into thioglycolate broth, incubated at 37°C for 24 to 48 hours, and then inoculated on blood agar. The infected medium was then anaerobically incubated for 3 to 7 days in an anaerobic jar created by an anaerogen gas pack **(16)**.

Identification of Bacterial Isolates

The Gram stain was conducted to identify the bacteria, and the Gram-negative ID kit (Biomerieux, France) was used for the validation of the results using the automated microbiological Vitek2 system **(17)**.

Isolates Morphological Characterization of Bacteria

Smears from recently formed colonies were colored with Gram stain, cultured on blood agar, and examined under a microscope to observe the dye reaction with the organization and structure of the colonies. Blood agar was used to cultivate the isolates, and their colonies were characterized based on factors such as form, pigmentation, edge, and color change **(18)**.

Molecular Assay

The obtained bacterial colonies were injected into the nutrient broth and grown for a full day or two at 37°C. DNA was extracted from the bacterial isolates using Geneaid Kit (Taiwan, Korea) as per the manufacturer's instruction. The isolates were identified using PCR reaction by amplifying universal 16S rDNA by specific primers: F 5'-GTACAGTTGCTTCAGGACGTATC-3 and R 5'-GGTTACCTTGTTACGACTT-3' as well as two specific primers to identify bacterial isolates *P. gingivalis*: F1 5'-AGGCAGCTTGCCATACTGCG-3 and R1 3'-CTGTTAGCAACTACCGATGT-5'. The PCR products were assessed by running on agarose gel.

Drug Resistance Evaluation

The antibiotics Cefoxitin (fox) 30mcg, Doxycycline (Do) 30mcg, Ciprofloxacin (CIP) 5mcg, and Nalidixic acid (NA) 30mcg were tested against *Porphyromonas gingivalis* using disc diffusion method.

3. Results

The periodontitis samples inoculated into liquid thioglycolate were assessed and diagnosed morphologically and microscopically. From 100 bacterial isolates, 35 showed significant positive growth and turbidity formation. *Porphyromonas gingivalis* produced black and rod-shaped colonies following 7 days

incubation on blood agar plates, which are attributed to the production of hemin from erythrocytes. The result is shown in Figure 1.

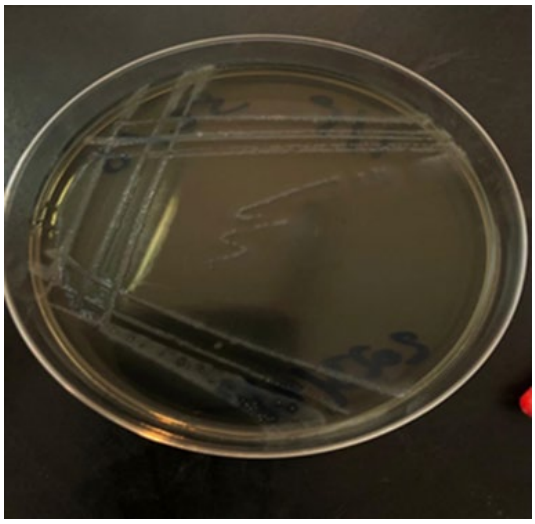

Figure 1. *Porphyromonas gingivalis* grown on blood agar

Extracting DNA

Genomic DNA recovered from the isolated bacteria was identified using agarose gel electrophoresis (Figure 2).

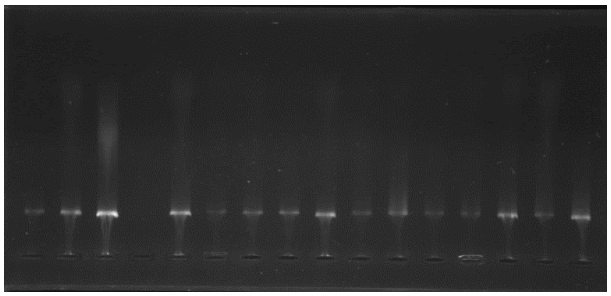

Figure 2. The genomic DNA bands of bacterial isolates on agarose gel.

Amplification using Specific Primers

The collected DNA samples from the identified microorganisms were successfully amplified by PCR using specific primers. In order to determine which primers were amplified, the PCR products were run on agarose gel. Compared to the standard molecular DNA marker, which is between 100 and 300 bp, the individual genes were differentiated by 150 bp, as shown in Figure 3.

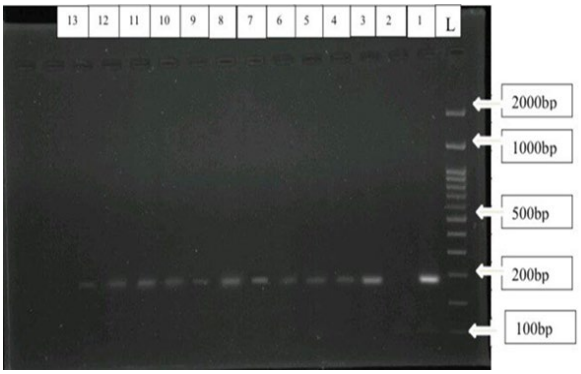

Figure 3. PCR products (150 bp) amplified by specific primers. L: Ladder

Virulence genes: Fimbria (fim)

Two types of Universal primers were used. Using specific primers, all 26 newly identified bacteria containing fimA gene were amplified and confirmed by running on agarose gel (234 bp) (Figure 4).

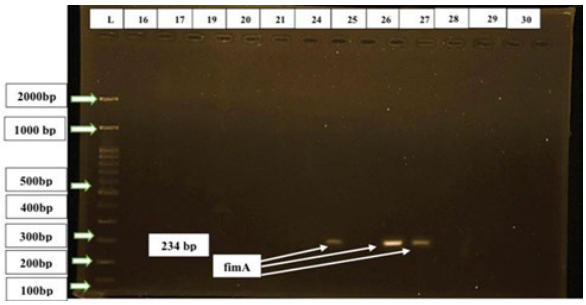

Figure 4. PCR amplified fimA gene (234 bp) of *Porphyromonas gingivalis* using specific primers.

Drug Resistance Result

Disc diffusion method showed Gram-negative anaerobic isolates resistance to all used antibiotics *in vitro*.

4. Discussion

The efficiency of the thioglycolate has been shown as carrier media in transporting and maintaining the vitality of *Porphyromonas gingivalis* from the mouth until it is delivered to the laboratory and cultured. The primary isolation media and solid development media are important in supporting the growth of these bacteria colonies which are crucial for their treatment plans **(19)**.

In this study, each aliquot that was used for the culture was supplemented with hemin and then plated onto blood agar. Following storage of the plates anaerobically in the jar equipped with a specially designed gas pack system for seven days **(20)**, the black-pigmented aggregates showed up. The black dye is created as a result of hemin interacting and

supporting iron movement **(21)**. This may help to explain why individuals with high iron intake are more likely to develop gingivitis.

Hemin is used by *P. gingivalis* as a source of iron for growth, resulting in the production of black pigments and the enlargement of plaques in the dental tissue and gums. Following the incubation time, the plates were checked for the presence of any tiny, glossy, coccobacilli, black-pigmented, and mucoid colonies (Figure 1).

Nalidixic acid is a synthetic quinolone antibiotic that acts as a bactericidal agent. About 20% of *Porphyromonas gingivalis* isolates were found to be resistant to Nalidixic acid, similar to previous reports. Ciprofloxacin is a fluoroquinolone antibiotic effective against most Gram-negative and Gram-positive bacteria. Cefoxitin is a second-generation cephalosporin antibiotic that protects against anaerobic bacteria. It showed resistance in 13% of *Porphyromonas gingivalis* isolates. Doxycycline is a broad-spectrum antibiotic belonging to the tetracycline family and fights germs by preventing their multiplying. It showed resistance to 26.6% of *Porphyromonas gingivalis* isolates, consistent with previous studies **(20)**.

According to the research conducted by Ingalagi *et al.* **(22)**, it is beneficial to employ several techniques to identify and quantify *P. gingivalis* and other periodontal infections in plaque samples **(23)**. These colonies can be small, glossy, black-pigmented, mucoid, and with or without hemolysis.

Porphyromonas gingivalis bacterial isolates were successfully detected using specific primers that cover the upstream regions of the 16S rDNA **(24)**. Investigation revealed that the PCR approach to identify bacteria with specific primers is unique to target bacteria but is limited to detecting the DNA of the target bacterium and is not suitable for the qualitative analysis of many unidentified bacterial species **(25)**. Several studies have shown that using conventional culture techniques are not suitable for the obligatory anaerobes as they are rarely identified in the root canal of the patients with periapical periodontal disease **(26)**.

The 16S rRNA gene detection by PCR helps to identify a variety of anaerobic bacteria that are resistant to growth in conventional cultures. The outcomes are comparable to the ones published before **(27)**.

Fimbriae, major virulence factors in *P. gingivalis*, play a significant role in the colonization process and facilitate attachment to other oral bacteria and host cell **(28)**. They are present in various commensal and pathogenic Bacteroides species in the human microbiota. A population-based study found that *P. gingivalis* has diverse occurrence patterns at the level of its virulence genes, and various factors influence its prevalence **(28)**. PCR tests with specific primers can reliably identify the presence of *P. gingivalis* and essential virulence factors, such as fimA, which shows its potential use in the context of periodontal disease **(29)**. The detection of fimA, prtC, IktA, and fap as virulence factors are substantially correlated with increasing in the frequency of target pathogens like *P. gingivalis* detection.

People of all ages can develop periodontitis with inflammation of the tissues; however, it is more common in those between the ages of 31 and 70 **(30)**.

5. Conclusion

Porphyromonas gingivalis is the primary causative agent of gingivitis. It was discovered that the genes of Fimbriae, particularly the two types (Fim A and Fim B of *P. gingivalis*) are among the most significant factors that promote the disease of gingivitis and periodontitis, according to the molecular analysis. The study highlights the need for innovative medicines to treat oral infections due to antibiotic resistance and side effects.

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Ethics approval

This study was approved by the Ethics Committee of the College of Medicine, University of Misan (Ethical code No. 198, 22/11/2022).

Conflict of Interest

The authors declare no conflict of interest.

Authors' contribution

Zhraa F. Faruq designed and performed the experiments and analyzed the data. Sami Kh. Jabar assisted in writing and revision of the manuscript with Dr. Mohammed Abas Abid Ali consultation.

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