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Genetic Diversity and Local Prevalence of *Porphyromonas gingivalis* and *Prevotella intermedia* Among Periodontal Patients in Diwaniyah, Iraq

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Abstract

This study aimed to investigate the prevalence of *Porphyromonas gingivalis* and *Prevotella intermedia* in gingivitis and periodontitis patients in Diwaniyah, and to analyze their virulence genes (fimA and adpC) using real-time PCR and sequencing. A total of 120 clinical isolates were obtained from patients aged 16–65 years with suspected gingivitis or periodontitis. Samples were analyzed by quantitative real-time PCR for detection of *P. gingivalis* and *P. intermedia*, followed by sequencing of fimA and adpC genes for phylogenetic analysis. Statistical tests (Pearson correlation and ANOVA) were used to evaluate associations between bacterial loads, clinical parameters, and demographic factors. *P. intermedia* was detected in 78.33% of patients, while *P. gingivalis* was present in 37.5% . Sequencing revealed significant genetic diversity among isolates, with multiple genotypes identified for both species. Phylogenetic analysis indicated local clustering of some strains, while others formed independent evolutionary lineages, suggesting possible new variants. The high prevalence of *P. intermedia* compared to *P. gingivalis* highlights its prominent role in the studied population. Genetic heterogeneity of virulence genes suggests ongoing bacterial adaptation, which may contribute to disease severity. Real-time PCR and molecular typing provide reliable tools for early detection and epidemiological tracking of periodontal pathogens.

Keywords: Gingivitis - *Porphyromonas gingivalis* - *Prevotella intermedia* -(qPCR) - fimA gene - adpC gene

Introduction

Gingivitis is the mild form of inflammation limited to the gingival tissue, which affects nearly 90% of the population, and can be reversed when the microbial deposits are eliminated (1). However, in vulnerable people with risk factors that impair the immune system, gingivitis can develop into periodontitis, which results in the irreversible loss of alveolar bone and clinical attachment.It is accounted for by the persistent inflammation in the periodontal tissues that arises from the host immune system's damaging reaction to ecological perturbations that alter the dysbiotic microbes (2).

Understanding the compositional transition in the oral microbiome from a healthy state to gingivitis and periodontitis has been of ongoing interest (3). Clarifying certain microbial problems linked to the onset and progression of periodontal disease is essential for early diagnosis and prevention, even if a number of factors influence the host response. Periodontal diseases, particularly gingivitis and periodontitis, represent An important worldwide health burden because of their high frequency and influence on quality of life (4).

These inflammatory conditions not only impact oral health but have also been increasingly associated with various systemic diseases including cardiovascular disorders, and diabetes (5).The pathogenesis of these conditions is fundamentally linked to dysbiosis of the subgingival microbiome, where certain bacterial species transition from commensal organisms to pathogens (6).

Among the key periodontopathic bacteria, *Porphyromonas gingivalis and Prevotella intermedia* have been extensively implicated in disease initiation and progression through their sophisticated virulence mechanisms (7). These Gram-negative anaerobic bacteria produce an array of virulence factors, including fimbriae, proteases, and toxins, that enable host tissue colonization, cellular invasion, and immune evasion, ultimately leading to the destruction of periodontal supporting tissues (8). *P. gingivalis*, often termed a "keystone pathogen," possesses an impressive arsenal of virulence factors including fimbriae, gingipains, and lipopolysaccharides that enable it to subvert host defenses and manipulate the oral microbiome (9). The fimA gene in P. gingivalis encodes the major fimbrial subunit, which is crucial for initial attachment to host cells and biofilm formation (10). Similarly, the adpC gene in P. intermedia encodes an important adhesin protein that contributes to subgingival colonization and modulation of host immune responses (11).

Advanced molecular techniques, particularly reaction (qPCR), have become indispensable tools for accurate detection and quantification of periodontal pathogens, offering superior sensitivity and specificity compared to traditional culture-based methods (12) . This study employed qPCR technology to investigate the prevalence and distribution of these important pathogens, their association with demographic factors, and the detection of their primary virulence genes in samples from gingivitis patients in the Diwaniyah population.

Materials and Methods

Sample Collection

One hundred and twenty dental paper points were collected, distributed into two groups (66 females and 54 males). Patients were clinically diagnosed by a dentist as having gingivitis with red and bleeding gums, pain, pocket discharge, and bad breath. The patients, aged 16-65, visited private dental clinics in Diwaniyah between February 2025 to August 2025. Each patient's name, age, gender, medical condition, and sample collection date were recorded, and the sample was transported to the laboratory for preservation.

Sequencing of PCR Products

All PCR products obtained above were purified and sent for sequencing as follows:

1. After PCR amplification , noting that the target region of the fimA gene is 514 base pairs long using the new primers, the PCR product was purified from amplification primers using the Gel/PCR DNA Extraction Kit (Geneaid, USA).
2. The purified DNA was sequenced at Macrogen (Korea) using sequencing primers for each gene as listed in Table (8-3).
3. Sanger sequencing was performed in both directions using an Applied Biosystems 3730 xl DNA Analyzer.

Table (1): Sequencing Primers Used in This Study

Reference	Product Size (bp)	Sequence 3_5	Primer Name	Gene
Current Study	278	ATGAGGTTGAGGCCTTGACG	FimA-F	**FimA** **P.gingivalis**
		GGCTGCGATTTTAGCGTCAG	FimA-R	
Current Study	584	CACAAGCAAACGCACTCGAA	adpC-F	**adpC** **P.intermedia**
		CTGCCAACGGGTAAGCTACA	adpC-R	

Statistical analysis

Data were collected, compiled, analyzed, and presented using Microsoft Office Excel 2010 and the Statistical Package for Social Sciences (SPSS) version 25. The chi-square test was used to determine if any two category variables were related. P-values under 0.05 and 0.01, respectively, were considered to be at the highly significant level of significance.

Result and Discussion

Investigation of bacterial distribution

The present study included 120 clinical isolates from patients with a suspicion of gingivitis or periodontitis during the period from February 2025 to August 2025. The subjects varied in age between 16 and 65 years (mean: 36.77). *P. gingivalis* was detected in 37.5% (n = 45) of samples and *P. intermedia* in 78.33% (n = 94) by molecular analysis using real-time PCR. These results are visualised in Figure 1 and summarized in Table 2.

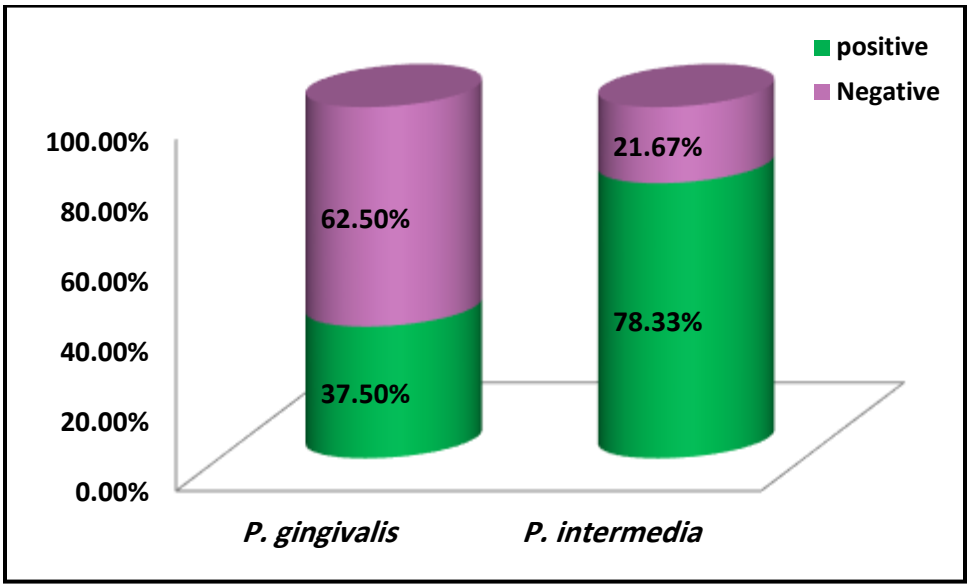

Figure 1: Detection rates of *P. gingivalis and P. intermedia* in clinical samples.

This bar chart illustrates the proportion of patient samples (total = 120) in which *P. gingivalis* and P. intermedia were identified through qPCR analysis. Notably, *P. intermedia* was present in a much higher percentage of cases (78.33%) compared to *P. gingivalis* (37.5%), suggesting that *P. intermedia* might participate in a more prominent part in periodontitis among the studied population.

X-axis: Bacterial species (*P. gingivalis*, *P. intermedia*), Y-axis: Detection rate (%)

Table 2: Relative distribution of bacteria detected in patients with gingivitis and periodontitis by qPCR analysis

Result	Number	Percentage	Number	Percentage
Positive	45	37.5%	94	78.33%
Negative	75	62.5%	26	21.67%
Total No	120	100%	120	100%
p value	0.006*		0.001*	

Figures 2 and 3 show the amplification plots of real-time PCR for *P. gingivalis* and *P. intermedia*, respectively. These results demonstrate a positive standard curve to correlate with patient copy numbers (exponential amplification phase) and assay robustness.

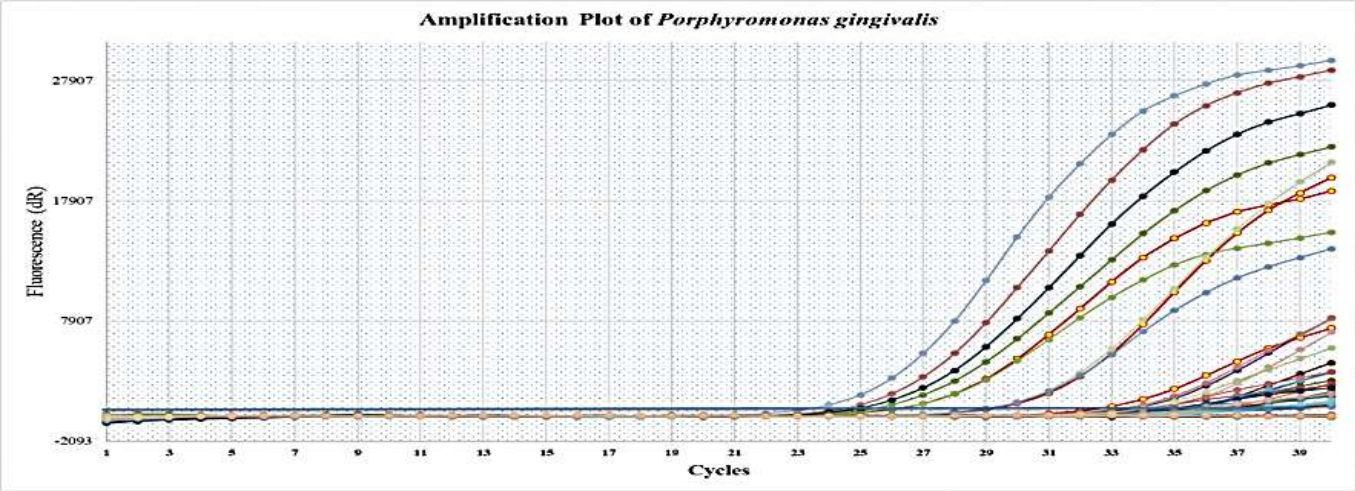

Figure 2: Amplification plot for *P. gingivalis* detection by real-time PCR.

This amplification plot illustrates real-time PCR analysis results of *P. gingivalis* in 120 clinical samples. Standard curve dilutions from 10-fold serial dilution of positive control DNA were taken as a reference for precise approximation of bacterial copies. Curves for the patient samples are plotted independently, with the exponential phase of amplification occurring. The "dR" signal is from the baseline subtracted fluorescence (ΔRn), which represents the difference in fluorescence due to DNA amplifcation.

X-axis: Cycle number (Ct), Y: ΔRn (change in fluorescent intensity), Legend: Curves represent patient sample; quantification is performed by means of standard curve points; negative controls have flat lines (nonamplification).

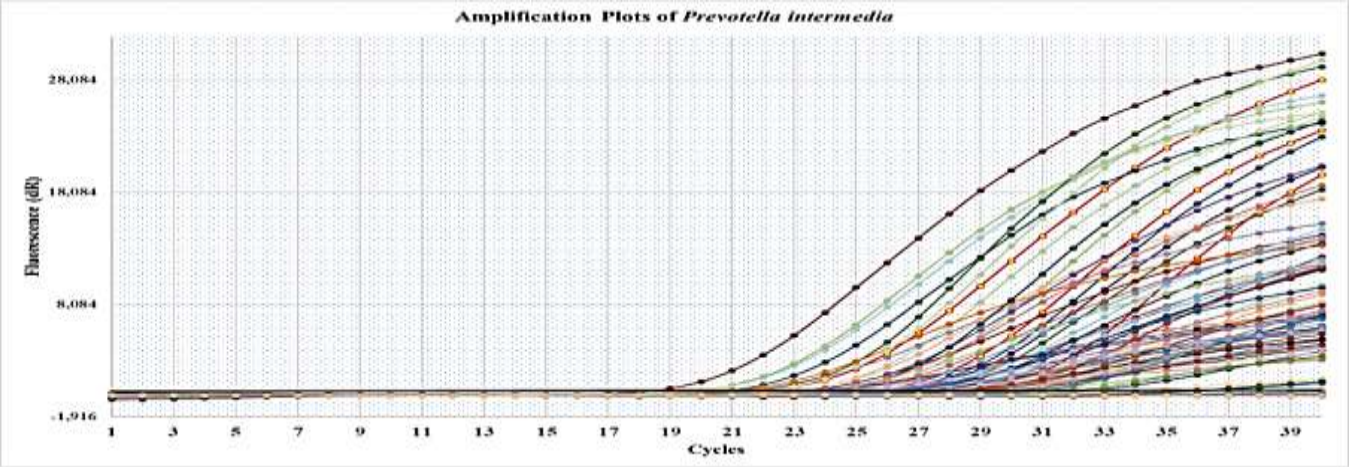

Figure 3: Amplification plot for *P. intermedia* detection by real-time PCR.

This plot displays real-time PCR amplification data for *P. intermedia* across 120 samples. A standard curve derived from decimal dilutions of known DNA concentrations was included to calculate copy numbers. Amplification curves show a broader distribution of Ct values, suggesting variation in bacterial load. The "dR" label denotes the ΔRn signal, representing fluorescence adjusted for background noise. X-axis: Cycle number (Ct), Y-axis: Fluorescence intensity (ΔRn), Legend: Patient samples, standard curve, positive control, and negative control are differentiated by color or symbol.

Phylogenetic Tree of *P. gingivalis* Isolates

The fimA gene sequences of *P.gingivalis* were analyzed, and an evolutionary tree was constructed using Mega11 version 11. The tree also included bootstrap values, which provide a measure of confidence at each branching point. High values indicate strong support for the relationships shown in the tree. These values are necessary to verify the reliability of evolutionary inferences and ensure that the observed branches are statistically reliable. The evolutionary tree showed clear variation between the isolates . They were classified into 8 main genotypes as follows: Genotype 1 includes isolates PG 42 and PG 45, while isolate PG 68 represents Genotype 2. Genotype 3 is represented by isolate PG 66, while Genotype 4 includes isolate PG 41. Genotype 5 includes isolate PG 64, while Genotype 6 includes isolates PG 11 and PG 24. Genotype 7 includes isolate PG 43, and finally Genotype 8 includes isolate PG 90. From the results, we note that Genotype 1 includes isolates PG 42 and PG 45, and Genotype 6 includes isolates PG 11 and PG 24, as they cluster together. On a common branch and close to the reference type of *P.gingivalis* isolate, indicating its genetic closeness to it. This homogeneity could be the result of local transmission or stability of strains in a limited area, while the remaining genotypes lie on an independent evolutionary branch, indicating the possibility of being a new species or distinct lineage. In the study of (13) Chen et al. they noted that the evolutionary tree of eight *P.gingivalis* isolates. Lineages 381, ATCC33277, and HG66 appeared closest to the likely common ancestor, based on the tree inferred from the presence of a close species as an outgroup sequence. While the other lineages gradually diversified to deeper branching nodes, the sequences of two lineages, F0566 and TDC60, were the most branched and mutated from the common ancestor, which was inferred using the sequence of a neighboring species (PaDSM20707) as an outgroup.

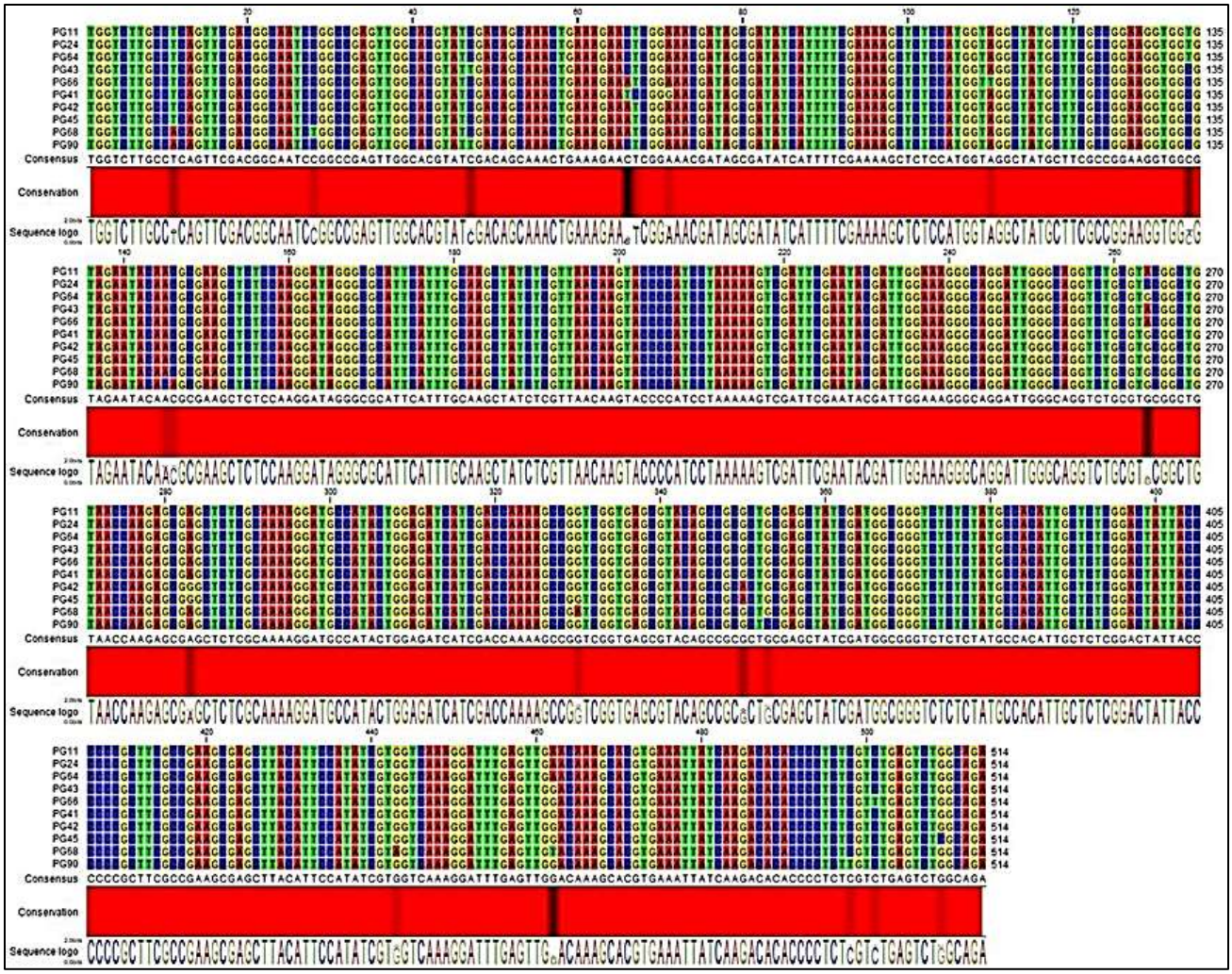

Figure (4) shows the regularity of the multiple FimA gene and detection of variants (base substitutions) for the studied isolates of *P. gingivalis.*

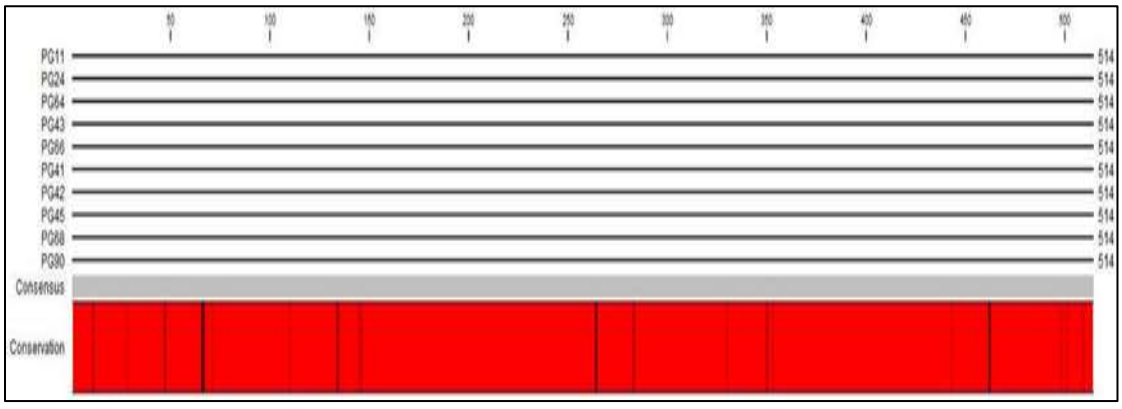

Figure (5) The gene summary shows the proportions of conserved and gap regions in the FimA gene among the studied *P. gingivalis* isolates. Note: The conservation color represents the similarity ratios between the studied samples.

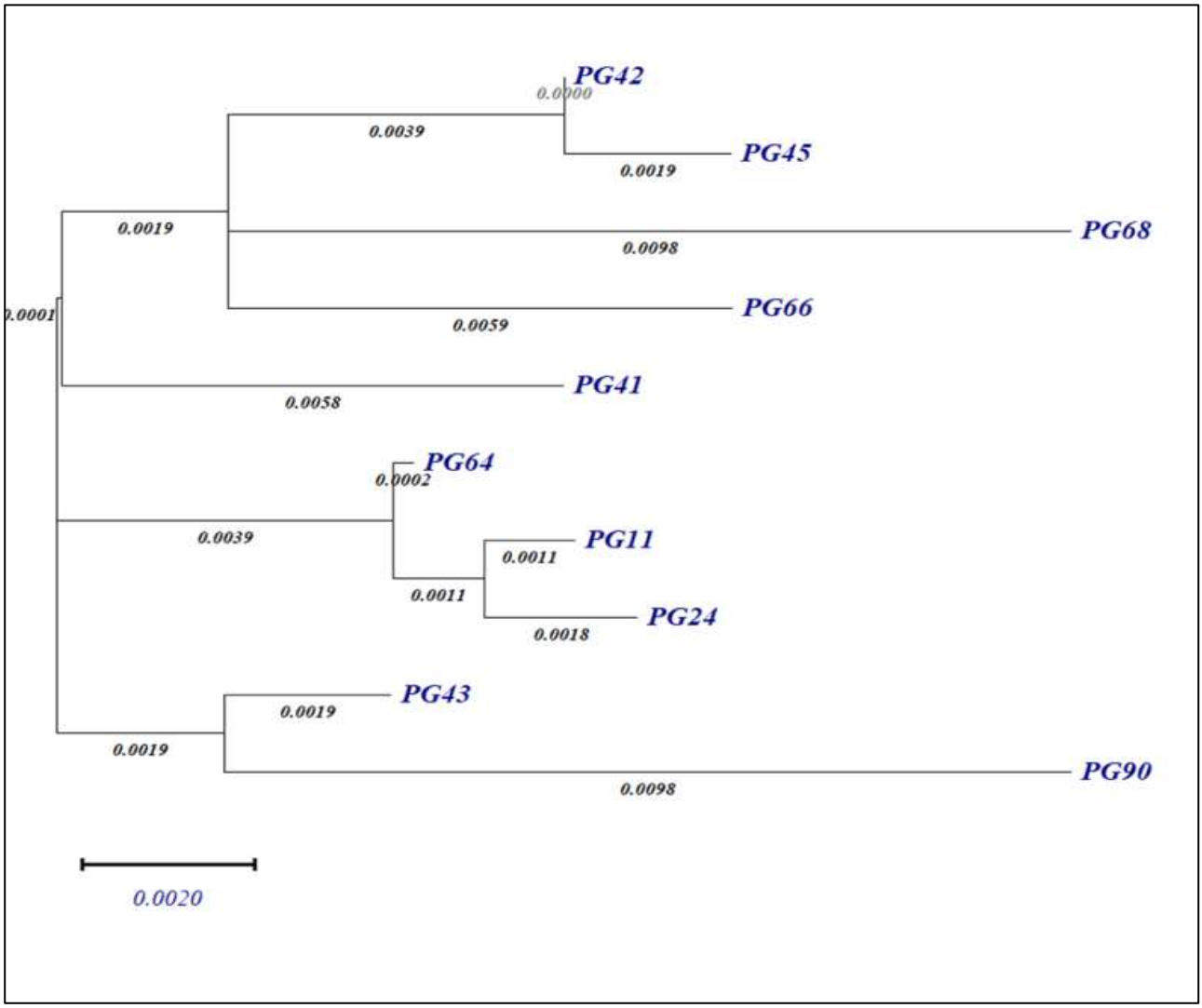

Figure (6) Schematic diagram of the evolutionary tree of *P. gingivalis*. This tree was constructed using the maximum likelihood method. Branch lengths for each sample were added to illustrate the distance between the studied isolates

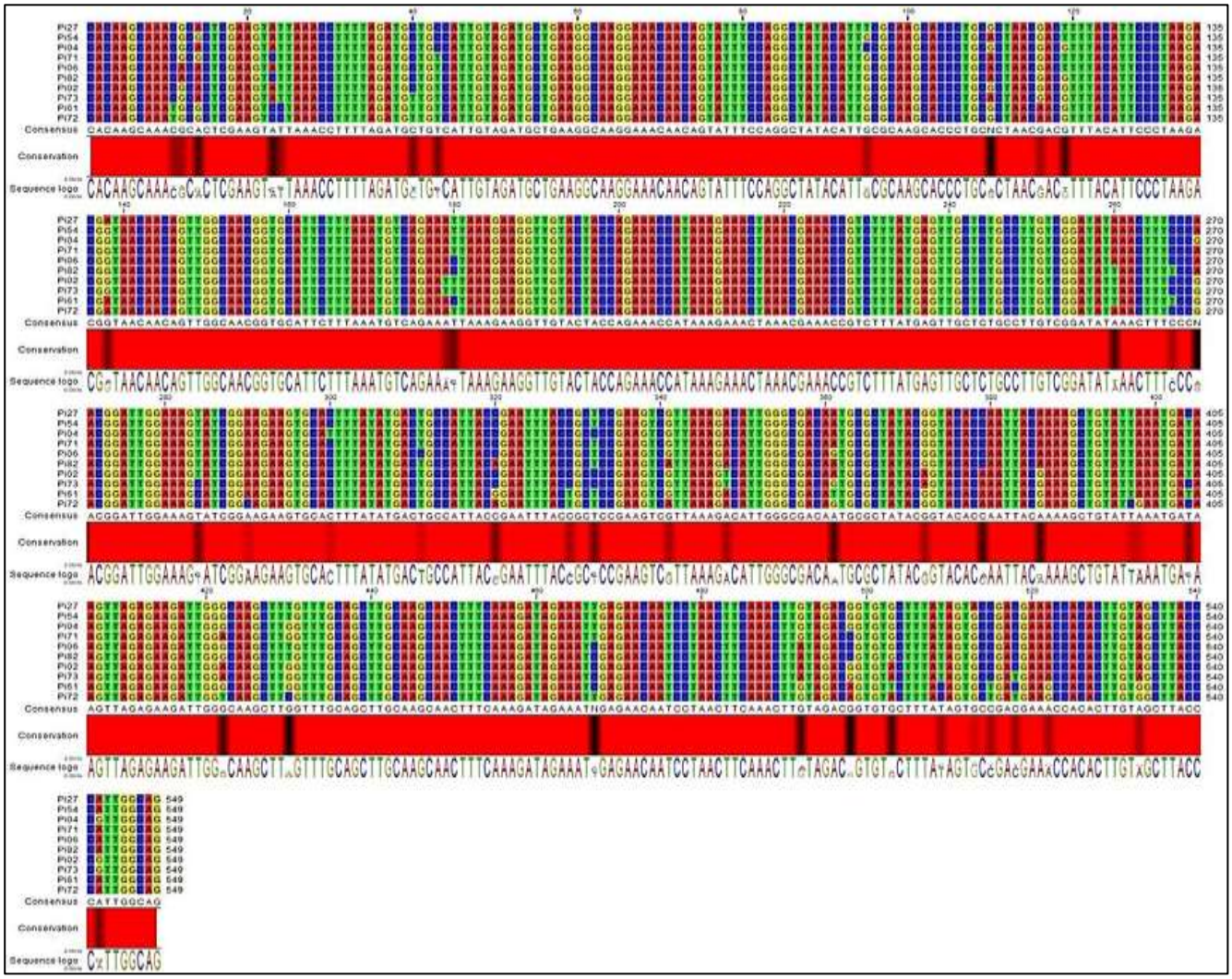

Figure (7) Regularization of the multiple FimA genes and detection of variants (base substitutions) for the studied isolates of *P. intermedia*

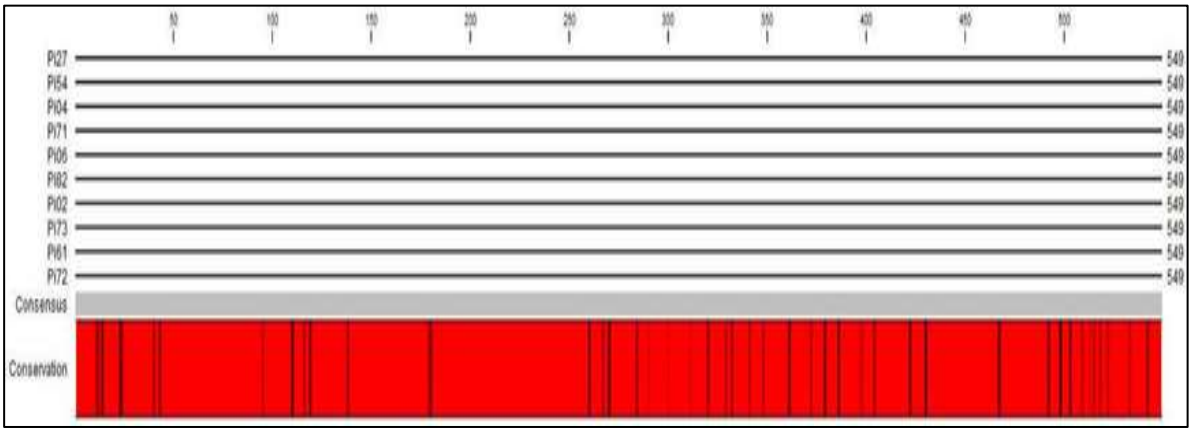

Figure (8) shows the gene summary of the proportions of conserved regions and gaps in the FimA gene among the studied *P. intermedia* isolates. Note: The conservation color represents

Phylogenetic tree of *P. intermedia* isolates

The AdpC gene sequences of *P. intermedia* were analyzed, and an evolutionary tree was constructed using the Mega11version11 program. The tree also includes bootstrap values, which provide a measure of confidence at each branching point. High values indicate strong support for the relationships shown in the tree. These values are essential for verifying the reliability of evolutionary inferences and ensuring that the observed branching's are statistically reliable. The evolutionary tree showed clear variation among isolates, and they were classified into seven main genotypes, as follows: Genotype 1 includes isolates Pi06 and Pi82, while isolate Pi71 represents Genotype 2; Genotype 3 is represented by isolate Pi54, while Genotype 4 includes isolate Pi27; Genotype 5 includes isolate Pi04, while Genotype 6 includes isolates Pi02 and Pi73; and Genotype 7 includes isolates PG61 and PG72. From the results, we note that Genotype 1, which includes isolates Pi06 and Pi82, Genotype 6, which includes isolates Pi02 and Pi73, and Genotype 7, which includes isolates PG61 and PG72, cluster on a common branch close to the reference type of *P. intermedia*, indicating their genetic closeness. This homogeneity could be the result of local transmission or stabilization of strains in a limited area, while the remaining genotypes lie on an independent evolutionary branch, indicating the possibility of them being a new species or distinct lineage. The evolutionary divergence of these three bacterial species was studied through comparisons of the *16 rRNA* sequences of the (rrs) gene of the epidemiologically and geographically diverse *P. nigrescens, P. intermedia,* and *P. gingivalis* isolates. The divergence was also lower than in *P. intermedia,* with a maximum of 13 out of 1363 nucleotides diverging between different strains, most of which exhibit ambiguous nucleotides. This genetic diversity detected among isolates reflects the presence of clear differences in genetic composition. It may be due to environmental adaptation or diversity of the source of isolates.

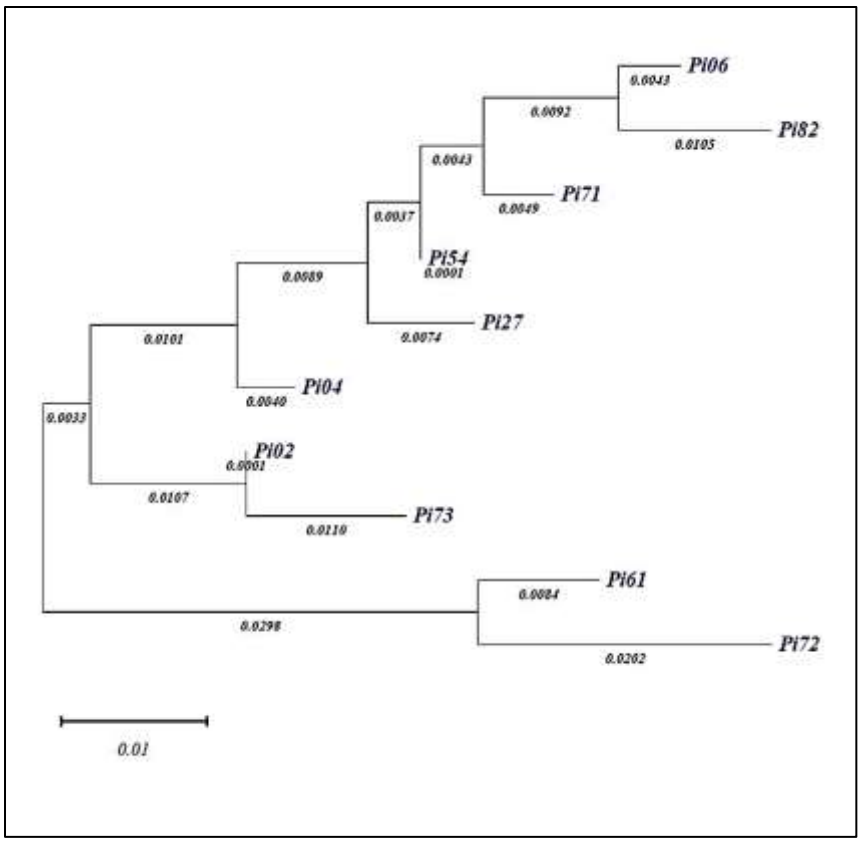

Figure (9) Schematic diagram of the evolutionary tree of *P. intermedia*, This tree was constructed using the maximum likelihood method, and the branch lengths of each sample were added to illustrate the distance between the studied isolates

The findings agreed with those of Papone *et al*., study of 51 chronic periodontitis patients. *A. actinomycetemcomitans* (33%) and black-pigmented anaerobic bacteria (100%) were found in the samples according to the results of the conventional microbiological technique, but by using multiplex PCR, we were able to determine that the most common species were *F. nucleatum* (100%), *T. forsythia* (92%), and *P. gingivalis* (88%) (14). *A. actinomycetemcomitans* (33%) and *P. intermedia* (39%) were the least common species, on the other hand. The findings also concurred with Alazemi *et al*., who discovered that *P. gingivalis* was isolated from individuals with periodontitis less frequently (33.3%) than other periodontitis-causing bacteria (15).

In a research including 30 individuals with peri-implant disease (14 men and 16 women), ages 35 to 60 (mean age 45.8 years), 26 samples from gingival and peri-implant samples were identified as belonging to 7 *P. gingivalis* and 19 *P. intermedia*. Four P. intermedia strains were found in only four samples (5.48%) out of the 73 peri-implant samples. There were no identified strains of *P. gingivalis*. Nine samples (12.33%) of the 73 gingival samples also contained only 15 strains of *P. intermedia*. Seven strains of *P. gingivalis* were found in one sample (1.37%) (16). The most prevalent periodontal pathogens in investigations of Brazilians with periodontitis were *P. medium* and *F. nucleatum*, which had a 67% detection rate. *A. actinomycetemcomitans* (13%) and *T. forsythia* (20%) were less common species *than P. gingivalis* (26.7%) (17).

In a study of the isolation of bacteria causing gingivitis in patients with oropharyngeal cancer, significant variations were found for *P. intermedia*, which was present in 83.3% (20/24) of patients. *P. gingivalis* was identified in 66.7% (16/24) of patients, and *T. forsythia* was present in 41.7%. (10/24) of patients, as these results showed that the three periodontal pathogens were more prevalent in patients compared to healthy people (18).

A considerable microbial diversity was shown by the description of over 700 bacterial species in the mouth cavity. Dental caries and periodontal disease, which are quite common in adults, are the primary results of oral cavity disorders associated to a bacterial imbalance, according to Chattopadhyay et al., (19).

However, in terms of identifying periodontal infections in saliva samples, the result was different from Choi et al., Prior to *P. anaerobius, P. gingivalis, E. corrodens, C. rectus* and *T. denticola* . the most prevalent bacterium was *F. nucleatum,* which differed significantly from all other bacteria. The copy numbers of DNA were Compared to the microorganisms listed above, the prevalence of *P.intermedia, T.forsythia* and *A.actinomycetemcomitans* is significantly lower (20). Different sample and DNA extraction methods may contribute to the variation in proportions between studies, but other factors like regional, ethnic, etiological, genetic, environmental, and dental hygiene habit differences may also play a role (15).

The findings were in line with those of Mahdi *et al*., who demonstrated that out of thirty samples from patients receiving endodontic treatment, only ten isolates (33.3%) of *P. intermedia* emerged by culture and biochemical testing, compared to six isolates (20%) that emerged during molecular detection (21) . This result is nearly in line with the research by Milsom et al., which found that the polymerase chain reaction likewise demonstrated P. intermedia detection rates of 33% greater than the culture method (13%) (22). However, Riggio *et al*. discovered that 38 samples (39%) of the 97 samples examined by the polymerase chain reaction tested positive for *P. intermedia* in subgingival plaque samples from individuals with adult periodontitis (23) .

The distribution of *P. intermedia* and the levels of pathogens in the gums in saliva samples from infected individuals compared to healthy controls, however, exhibited higher mean levels in individuals with periodontitis and significantly different levels for *P. gingivalis*, *P. intermedia,* and *T. forsythia*, according to multiple studies. Each of these bacteria had mean levels that were up to 20 times greater than those in the uninfected group (24). According to a study by Castañeda-Corzo and his colleagues (2023), *P. intermedia* was found in 83.3% of infected people compared to 25% of the uninfected group, which is a highly significant difference. *P. intermedia* is intimately linked to a number of disorders that impact numerous systems, according to recent research. *P. intermedia* may attach to and infiltrate a variety of host cells, including gingival fibroblasts, thanks to virulence factors such cysteine, proteinases, adhesives, and biofilm formation. This can make it easier for different germs to colonize. pathogenic, producing periodontal disorders and disrupting the host immunological inflammatory response (25).

Conclusions

This study demonstrated that *P. intermedia* is more prevalent than *P. gingivalis* among patients with gingivitis and periodontitis in Diwaniyah, indicating its potential as a major pathogenic contributor. The detection of multiple genotypes of fimA and adpC genes reflects significant genetic diversity, suggesting local adaptation and possible emergence of novel strains. These findings underscore the importance of molecular techniques in identifying periodontal pathogens, understanding their evolutionary patterns, and improving strategies for early diagnosis and targeted prevention of periodontal disease.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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