Mohsin *et. al.* Babcock Univ. Med. J.2025 8(1):26-42
https://doi.org/10.38029/babcockuniv.med.j..v8i2.939
eISSN: 2756-4657

RESEARCH ARTICLE OPEN ACCESS

Quantitative detection and virulence gene profiling of Prevotella intermedia and Porphyromonas gingivalis in periodontitis patients

Mohsin HQ[1], Al-Taweel RS[1ID]

[1]*Department of Biology, College of Education, University of Al-Qadisiyah, Iraq*

Submitted: 19th February 2025
Accepted: 26th May 2025
Published: 31st December 2025
ID: Orcid ID

Abstract
Objective: The present study was designed to determine the prevalence and detection of key virulence genes in individuals with gingivitis and periodontitis in Al-Diwaniyah Governorate and is one of the initial reports of fimA and adpC gene prevalence in Iraq.
Methods: Patients' age ranged from 18 to 68 years (mean = 36.77 years). Ninety clinical isolates were collected from these patients. The real-time PCR detected P. gingivalis and P. intermedia to calculate the number of bacterial loads [cycle threshold (Ct) values and copy numbers] and to detect the virulence factors fimA and adpC.
Results: The percentages of detection for P. intermedia and P. gingivalis were 82.22% and 34.44%, respectively. The differences were found independent of sex and age. The average age of infected participants was 36.47±8.67 and 39.16±8.31 years for P. intermedia and P. gingivalis, respectively. Cycle threshold values were 33.69±4.45 and 26.85±3.71, and loads were 533,612 and 745.95 copies, respectively. P. gingivalis was more abundant in subjects over 45 years, while P. Intermedia was more frequent in those over 65. The fimA gene was present in 60% of P. gingivalis isolates and adpC in 70% of P. intermedia.
Conclusion: The percentage of P. intermedia was higher than that of P. gingivalis in periodontitis patients. The higher bacterial load with age indicated that cumulative tissue damage is involved. fimA and adpC genes were also particularly informative as they represent potential diagnostic and therapeutic targets. High P. intermedia prevalence suggests its role as a primary pathogen in periodontitis.

Keywords: Periodontal diseases, P. intermedia, P. gingivalis, qPCR, adpC, fimA.

Plain English Summary

Gum diseases are strongly associated with disrupting the normal balance of bacteria and other microorganisms in the mouth. Prevotella intermedia and Porphyromonas gingivalis are most associated with the disease. The present study was designed to determine the prevalence and expression of microorganisms that have specified virulence in these individuals with inflammation of the gum and gum infection (periodontitis). Microorganisms were isolated from 90 patients during clinical investigation. Real-time PCR was used to detect real-time amplified DNA products. The percentage of P. intermedia was higher than that of P. gingivalis in patients with gum infection.

Introduction

Gingivitis is an infection of the soft tissue of the teeth triggered by the presence of pathogenic bacteria in the oral cavity. It is one of the commonest dental conditions and has the potential to progress to periodontitis. When it is

Correspondence:
Mohsin, Hodaa Q
Department of Biology, College of Education,
University of Al-Qadisiyah,
Iraq
+9647804402768, edu.bio.posta137@qu.edu.iq

not treated, the tooth is lost due to destruction of the supportive material around the teeth. It is an inflammatory condition that is heavily implicated in oral biofilm, which is a pathogenic biofilm that contains numerous bacteria and antigens, prompting chronic inflammation. Identification of the specific functions of these bacteria inducing this type of inflammation is important for the development of targeted treatments and prevention strategies (1).

There is that complex life community of bacteria and fungi, and viruses, and so on, in just a tiny drop of saliva. It is critical for oral and systemic health. The homeostasis of this ecosystem is influenced by diverse factors, among them the diet, tobacco, alcohol, way of life, and other diseases. Disturbance of this microbial homeostasis can cause several pathologies, including dental caries, gingivitis, oral candidiasis, and halitosis (2).

Gingival diseases, such as gingivitis, affect almost 50% of the global population at different levels. A severe form of gingivitis is found in 10–15 per cent of adults and is the greatest single cause of tooth loss. It generally occurs in adolescence and young adulthood and is often due to plaque accumulation along with poor oral hygiene. Notably, the prevalence of periodontal disease increases markedly with age, with 70%-80% of those older than 65 expressing at least a form of this disease (3).

Gingivitis-associated bacteria have virulence factors that affect their pathogenicity. Within the oral cavity, such virulence factors can be enzymes, e.g., proteases which degrade structural proteins, such as collagen in the gingiva, and endotoxins, such as LPS (lipopolysaccharides), inducing severe inflammatory reactions. Furthermore, these microorganisms can manipulate the host immune response or can be resistant to the detrimental inflammatory environment, thus providing a survival advantage and contributing to their pathogenic potential. The interrelationships and synergies between these pathogens continuously promote colonisation and detrimental consequences (4).

The quantitative distribution of bacterial species is proven to be a dependable marker for evaluating the degree of gingivitis. Accurate determination of the bacterial load within the tissues at the level of the individual patient may aid clinical staging of the disease and individualising treatment protocols. These analyses allow the tracking of the evolution of disease and the response to treatment (5). Polymerase chain reaction (PCR) has been used based on its high sensitivity for the determination of PG and PI DNA since it can identify small amounts of bacterial DNA, such as that found in P. intermedia (PI) and P. gingivalis (PG) (6, 7). This technique has also been used in Iraq to evaluate the long-term outcome of patients under treatment.

Lopes et al. developed a PCR technique for detecting the accumulation of three primary pathogens of periodontal disease, Actinobacillus actinomycetemcomitans, P. intermedia, and P. gingivalis. Their strategy was successful in defining the unique functions of these bacteria at different stages of gingivitis. It provided an insight into microbial interactions in periodontal pathology and highlighted the significance of such insights in the design of targeted therapeutic strategies (8). Hameed et al. studied the novel inflammatory pathway of P. gingivalis in chronic periodontal disease. Their results underscored the ability of the bacterium to disrupt microbial homeostasis in the oral cavity and foster an inflamed environment, promoting the growth of other pathogens. Strategies consisted of the production of enzymes with proteolytic action to destroy the gingival tissues and with immuno-evasion mechanisms. For example, LPS release led to the production of cytokines, including IL-1 and IL-6, which cause a greater degree of local inflammation and ultimately periodontal destruction (9).

Ruan et al. recognised P. intermedia as an important cause of diseases as gingivitis, periodontitis, and peri-radicular inflammation. From an infected root canal of a Chinese patient with periodontitis, the strain possesses certain genomic characteristics conducive to adaptability in various environments and virulence, thereby facilitating its long-term existence in habitats like the oral and respiratory tracts (10).

How et al. defined periodontal diseases as infectious-mediated inflammatory conditions primarily induced by the initial build-up of bacterial biofilms on the tooth surfaces. These biofilms are communities of bacteria that can cause a gradual loss of supporting tooth structure. Although the subgingival plaque can accommodate over 500 bacterial species, P. gingivalis has been regarded as a keystone pathogen in chronic periodontitis owing to its large number of virulence factors, which induce direct tissue destruction in the gingival tissue and/or disrupt host immune response (11).

Deo and Deshmukh highlighted the importance of the oral microbiome, which is the second most complex and diversity-rich ecosystem after the gut microbiome, and hosts around 100 species of bacteria, as well as fungi, viruses, and protozoa. The development of advanced genomic technologies, such as high-throughput sequencing and bioinformatics, makes it possible to perform a comprehensive analysis of microbial function and metabolism, advancing our ability to

study the pathogenesis and develop precision therapy (12). Abdulkareem et al. suggest interleukin-15 may be critically involved in the initiation and development of periodontitis. The structural heterogeneity of the biofilm matrix in dental plaque (i.e., densely covered microbial communities) requires a better understanding of host-microbe interactions other than simple elevations in plaque load. Diagnostic improvements have shown that gingivitis progression is more associated with these complex interactions (13).

Könönen et al. studied the ecological functions of oral early coloniser Prevotella species, the majority of which contribute to inflammation in oral tissues. P. denticola, P. tannerae, P. intermedia, and P. nigrescens were found to be involved in periodontal and endodontic infections, dental caries, and were reported to be related to systemic diseases such as head and neck cancers (14). Bielecki et al. showed that P. intermedia, like P. gingivalis HmuY, expresses heme-reactive proteins with distinct nucleotide sequences but is capable of binding heme in a coordinated manner with heme iron. Such adaptation might help in the acquisition of heme and allow the bacteria to survive in inflammatory locations. Such molecular detail might aid the development of diagnostic and therapeutic approaches that are more effective (15).

Recent investigations have consistently demonstrated the important role of Porphyromonas gingivalis and Prevotella intermedia in both initiating and advancing severe periodontitis, largely due to their strong capacity to induce inflammation and cause tissue destruction (16, 17). Although these microorganisms are recognised as clinically important, there remains a noticeable deficiency of molecular epidemiological data concerning P. gingivalis and P. intermedia within Middle Eastern populations. Of particular concern is Iraq, where unique regional microbial profiles may shape disease severity and influence therapeutic outcomes.

The main objective was to determine the relation of the presence of P. intermedia and P. gingivalis with the severity of gingivitis. Moreover, it attempted to measure these two types of bacteria in clinical samples to find out about their involvement in the pathogenesis and progression of gingival inflammation. Our hypothesis posits that P. intermedia will be more commonly found in older patients and that its prevalence, along with the presence of virulence factors such as fimA and adpC genes, will be associated with greater destruction of periodontal tissues.

Materials and methods

Sample Collection

Participants eligible for the study were adults aged between 18 and 70 years, all of whom had a clinical diagnosis of either chronic gingivitis or periodontitis. Individuals who had taken widespread antibiotics, received periodontal treatment within the last three months, or had widespread health conditions affecting the periodontium, such as diabetes or immunosuppression, were not considered eligible.

Assessment of gingival inflammation and periodontal disease severity was carried out by a qualified periodontist using the Community Periodontal Index (CPI) along with clinical measurements, including probing pocket depth (PPD) and clinical attachment loss (CAL). The classification of periodontitis adhered to the 2018 case definitions established by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP).

Ninety clinical oral samples were taken with sterile dental paper points from patients diagnosed with gingivitis at the dental clinics. Participants were 90 adults (46 females, 44 males) between the ages of 18 and 70 years who attended private dental clinics in Al-Diwaniyah during the period from April to August 2024. Clinical indices were measured in gum redness, bleeding, pain, and halitosis. The following information was recorded for both patients: demographic data and clinical profile (name, age, sex, systemic illness, and date of collection). The collected specimens were immediately transferred into a guided medium and stored under appropriate conditions before transport to the laboratory for molecular analysis.

DNA Extraction

Genomic DNA from the bacterial samples was isolated according to the manufacturer's instructions with the Geneaid Presto™ Mini gDNA Bacteria Kit (Geneaid, USA). In brief, the samples were framed from the suspension saline, and the supernatant was centrifuged and removed. The bacterial pellet was disrupted by GT buffer and Proteinase K with incubation at 60°C, then cryptic genomic DNA was released by GB buffer and heated at 70 °C. â The DNA and RNA pollution was re-cleansed by silica membrane with ethanol in the GD column. The column was washed with W1 and Wash Buffers several times before being air dried. At last, the DNA was eluted by preheated (65°C) elution buffer and stored at 20 °C for subsequent use.

DNA Quantification

The extracted DNA was quantified, and purity was measured by a NanoDrop™

spectrophotometer. The concentration of DNA was determined (ng/µl), and the purity was checked based on the absorbance ratio at 260/280 nm. A solution of 2 µl deionised water was measured in the spectrophotometer as calibration before each measurement. Preparation was carried out using 1 µL of each DNA sample, and the coefficient of variation was calculated, and the substrate was wiped clean between runs for accuracy. Samples with a 260/280 ratio close to 1.8 were referred to as high purity.

qPCR (Quantitative Real-Time PCR)

To establish the P. gingivalis and P. Intermedia bacteria count in gingival samples, quantitative PCR (qPCR) was performed via commercial kits: GPS Preint detect-qPCR F100 and PorGin detect-qPCR F100 (Genetic PCR Solutions, Spain). These kits included species-specific primers and probes. Amplified products were generated in 20 µl reactions on a Stratagene Mx3005P Real-Time PCR machine (Agilent Technologies). The amplification conditions, as well as the reagent composition, are listed in Table 1.

Table 1: Thermal cycling conditions for qPCR analysis of *P. gingivalis* and *P. intermedia*.

Step	Temperature	Time	Description
Initial activation	95 °C	2 min	Enzyme activation
Denaturation (×40 cycles)	95 °C	5 sec	DNA denaturation
Annealing/Extension	60 °C	20 sec	Primer hybridisation and fluorescence detection

Fluorescent signals were read through FAM and HEX channels for target DNA and internal controls, respectively.

The positive control was diluted for standard curves with decimal dilutions to be used for copy number determination, fitted according to a linear regression equation between Ct values and log10 of the copy number:

1. Ct = Y-intercept + (Slope × log_{10} (Copy number))

2. Copy number = 10^ [(Ct - Y-intercept) / Slope]

Additional wells containing DNase/RNase-free water were included as negative controls for the reaction specificity in each run.

For each qPCR test, positive controls, using reference strains of P. gingivalis and P. Intermedia, were included, along with no-template negative controls to confirm the specificity of amplification. Standard curves constructed from serial dilutions of known DNA concentrations were employed to determine bacterial loads, with amplification efficiency and R^2 values carefully monitored to ensure accuracy.

Detection of Virulence Genes

The genes encoding a virulence factor (fimA for P. gingivalis, adpC for P. intermedia) were screened by conventional PCR. The procedure for DNA extraction was as follows, with that used for clinical isolates described above. The primers used were primer pairs designed from sequences available from the NCBI GenBank and synthesised commercially. The details of the primers are shown in Table 2.

Table 2: Primer sequences used for virulence gene detection

Gene	Primer Name	Sequence (5′–3′)	Product Size (bp)
fimA	FimA-F	ATGAGGTTGAGGCCTTGACG	278
	FimA-R	GGCTGCGATTTTAGCGTCAG	
adpC	adpC-F	CACAAGCAAACGCACTCGAA	584
	adpC-R	CTGCCAACGGGTAAGCTACA	

PCR Amplification

The reaction mixture (25 µl) was composed of GoTaq G2 Green Master Mix (Promega, USA), containing 12.5 µl master mix, 2.5 µl forward primers and reverse primers each, 3 µl DNA template, and 4.5 µl nuclease-free water. Amplification was performed with the following thermal-cycling profile (Table 3).

Table 3: PCR conditions for the detection of *fimA* and *adpC* genes

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	5 min	1
Denaturation	94 °C	1 min	35
Annealing	58 °C	1 min	35
Extension	72 °C	1 min	35
Final extension	72 °C	10 min	1

Gel Electrophoresis
The products were separated on a 2% agarose gel in the presence of Green Star™ dye. The gel was electrophoresed at 100 V for 36 minutes. The bands were visualised under a UV light with a Dual LED Blue/White Transilluminator (Bioneer, Korea), and the product sizes were estimated with a 100 bp DNA ladder.

Statistical Analysis
All statistical analyses were performed using IBM SPSS Statistics v23. The data were expressed in frequency (%), Mean ± SD. Pearson correlation tests were performed, and the significance level was set at $p < 0.05$ and $p < 0.01$. The Pearson correlation coefficient was used to evaluate the relationship between bacterial loads and clinical indices, and clinical periodontal indices, given that these variables were continuous and exhibited a normal distribution. To compare bacterial quantities across different age groups, a one-way ANOVA was conducted, with a significance threshold set at $p < 0.05$. Pearson's correlation coefficient was employed to examine the linear relationship between bacterial load and clinical periodontal indices, given that these variables were continuous and exhibited a normal distribution. To compare bacterial quantities across different age groups, a one-way ANOVA was conducted, with a significance threshold set at $p < 0.05$.

Results
Investigation of bacterial distribution
The present study included 90 clinical isolates from patients with a suspicion of gingivitis or periodontitis during the period from July 2024 to January 2025. The subjects varied in age between 18 and 68 years (mean: 36.77). P. gingivalis was detected in 34.44% (n = 31) of samples and P. intermedia in 82.22% (n = 74) by molecular analysis using real-time PCR. These results are visualised in Figure 1 and summarised in Table 4.

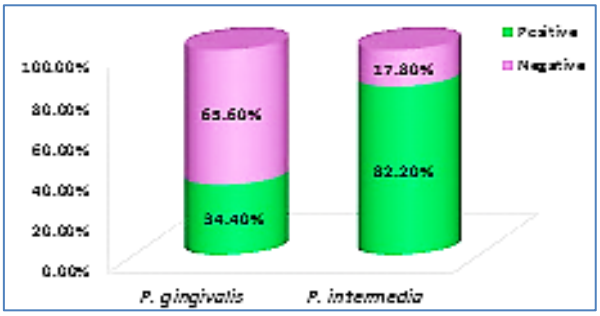

Figure 1: Detection rates of P. gingivalis and P. intermedia in clinical samples.\ X-axis: Bacterial species (P. gingivalis, P. intermedia), Y-axis: Detection rate (%)

This bar chart illustrates the proportion of patient samples (total = 90) in which P. gingivalis and P. intermedia were identified through real-time PCR analysis. Notably, P. intermedia was present in a much higher percentage of cases (82.22%) compared to P. gingivalis (34.44%), suggesting that P. intermedia may play a more prominent role in periodontitis among the studied population.

Table 4: Relative distribution of bacteria detected in patients with gingivitis and periodontitis by real-time PCR analysis

Result	Number	Percentage	Number	Percentage
Positive	31	34.4%	74	82.2%
Negative	59	65.6%	16	17. 8%
Total No	90	100%	90	100%
p value	0.003*		0.001*	

Demographic Characteristics of the Infected Patients
Demographic distribution of infection was examined and compared according to age and sex of the patients between the detection of P. gingivalis and P. intermedia. The average ages of patients infected with P. gingivalis and P. intermedia were 39.16±8.31 and 36.47±8.67 years, respectively. Statistical comparisons indicated no significant difference between groups for age (Table 5; p=0.292) or age category (Table 5; p=0.748). Regarding gender, P. gingivalis was detected in 38.7% of the male patients and 61.3% of the female patients, and P. intermedia in 51.4% of the males and 48.6% of the females. There were also no statistical

differences in these values (p = 0.237), as presented in Table 5.

Table 5: Demographic characteristics of patients with positive bacterial infections

Characteristics	*P. gingivalis*	*P. intermedia*	*P-value*
Age			
Mean ± SD	39.16±8.31	36.47±8.67	0.292
Range	22-68 years	20-68 years	
< 25, *n* (%)	4 (12.9 %)	14 (18.9 %)	0.748
25-45, *n* (%)	18 (58.1 %)	41 (55.4 %)	
≥ 45, *n* (%)	9 (29.0 %)	19 (25.7 %)	
Gender			
Male, *n* (%)	12 (38.7 %)	38 (51.4 %)	0.237
Female, *n* (%)	19 (61.3 %)	36 (48.6 %)	

*n: number of cases; SD: standard deviation; *: significant at P < 0.05.*

The prevalence of P. gingivalis and P. intermedia in general was 34.44% and 82.22%, respectively, using real-time PCR. These data are presented in Table 6 and were significantly different for both species (p<0.05).

Table 6. The relative distribution of *P. gingivalis* and *P. intermedia* in patients with gingivitis and periodontitis using real-time polymerase chain reaction (PCR)

Specie	Result	Number	Percentage
P. gingivalis	Positive	31	34.44%
	Negative	59	65.56%
	Total number	90	100%
	P value	P = 0.003*	
P. intermedia	Positive	74	82.22%
	Negative	16	17.78%
	Total number	90	100%
	P value	<0.0001*	

*Represents a significant difference at p ≤ 0.05 Data are expressed as mean ± standard deviation

Additional analysis of Ct values and bacterial copy numbers revealed that P. gingivalis has a mean Ct value of 26.85±3.71 and copy number of 745.95±2531.8, while P. Intermedia had a higher mean Ct of 33.69±4.45 and copy number of 533.612±1547.4 (Table 7).

Table 7. Comparison between the cycle threshold (Ct) and copy number of *P. gingivalis* and *P. intermedia* in the patient group

Specie	Result	Values
P. gingivalis	Cq	26.85±3.71
	Copy number	745.95±2531.8
P. intermedia	Cq	33.69±4.5
	Copy number	533.61±1547.4

**Represents a significant difference at p ≤ 0.05 Data are expressed as mean ± standard deviation*

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.

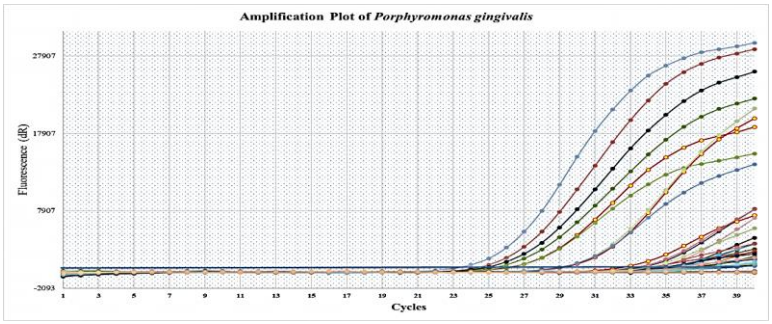

Figure 2: Amplification plot for P. gingivalis detection by real-time PCR

This amplification plot illustrates real-time PCR analysis results of P. gingivalis in 90 clinical samples. Standard curve dilutions from a 10-fold serial dilution of positive control DNA were taken as a reference for the 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 amplification.

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

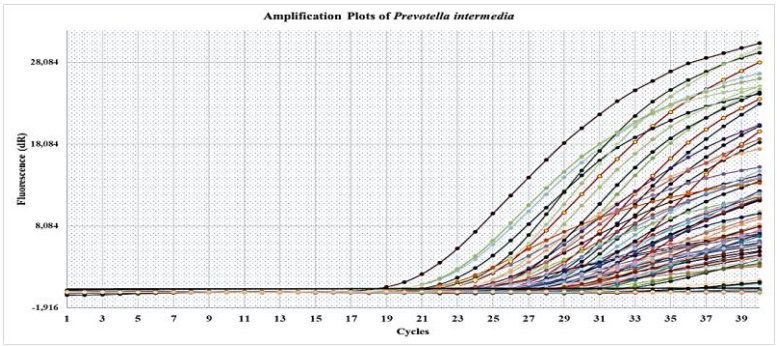

Figure 3: Amplification plot for P. intermedia detection by real-time PCR

This plot displays real-time PCR amplification data for *P. intermedia* across 90 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.

Gender Predominance of P. gingivalis and P. intermedia

Quantitative analysis of bacterial load according to gender, described in Table 8, did not produce statistically significant differences between the groups in the number of copies of P. gingivalis and P. intermedia. Average P. gingivalis copy number in males was 805.45±2214.3, and in females, 361.93±956.3. For P. intermedia, the mean CNV was 748.7±3059 for males and 742.9±1863 for females. The two species differed but not significantly in this regard ($p > 0.05$).

Table 8: Comparison of the cycle threshold (Ct) and the number of copies of bacteria by gender, *P. intermedia,* and *P. gingivalis*

Specie		Male (12)	Female (19)	p-value
P. gingivalis	Ct	32.59±4.64	34.39±4.3	0.293
	Copy number	805.45±2214.3	361.93±956.3	
P. intermedia		Male (38)	Female (36)	
	Cq	27.20±3.5	26.40±3.8	0.385
	Copy number	748.70±3059.0	742.90±1863.0	

**Represents a significant difference at $p \leq 0.05$ Data are expressed as mean ± standard deviation*

Age-Dependent Distribution of P. gingivalis and P. intermedia

The stratification of single-copy bacterial load by patient age showed age-correlated patterns in colonisation (Table 9). The mean value of P.

gingivalis count was highest in the >45 years of age group (954.9±2532), followed by the 65 years group (1002.13±2074), followed by the 45–65 years group (794.03±3089) and <45 years group (257.4±560.9), but the differences were not significant (p=0.437). These findings provide evidence for potentially accelerating bacterial accumulation with age, probably by inducing long-term periodontal tissue destruction or dysbiosis.

Table 9: Comparison Between Cycle Threshold (Ct) and Copy Number of *P. gingivalis* and *P. intermedia* According to Age

Specie	Age	Number	Gene expression	
			Ct	Copy number
P. gingivalis	<25	4		436.4±869.08
	25-45	18		344.6±969.9
	>45	9		954.9±2532
	P value		0.855	
P. intermedia	<45	14	26.17±2.4	257.40±560.9
	45-65	41	27.35±3.8	794.03±3089.0
	>65	19	26.26±4.2	1002.13±2074
	P value		0.437	

**Represents a significant difference at p ≤ 0.05 Data are expressed as mean ± standard deviation*

Distribution of Virulence Genes

To further explore the distribution of virulence genes across demographic groups, the positive samples for fimA and adpC were categorised by sex and age. Although the sample size for gene detection was limited to ten isolates per species, this analysis provides preliminary insight into possible patterns of gene prevalence. The findings are summarised in Table 10.

Table 10 Prevalence of virulence genes (*fimA* and *adpC*) by sex and age group

Gene	Group	Positive Cases	Total Cases	Prevalence (%)
fimA	Male	3	5	60
fimA	Female	3	5	60
fimA	<45 years	2	4	50
fimA	â‰¥45 years	4	6	66.7
adpC	Male	4	5	80
adpC	Female	3	5	60
adpC	<45 years	3	4	75
adpC	â‰¥45 years	4	6	66.7

A total of twenty isolates, comprising ten and ten for P. gingivalis and P. intermedia, respectively, were randomly chosen from the samples that tested positive via qPCR. This selection aimed to ensure both species were adequately represented, assisting the subsequent conventional PCR detection of virulence genes while maintaining practicality within laboratory constraints. The result of virulence genes expression among the isolates showed that the fimA gene was detected in 60% of P. gingivalis-positive samples and the adpC gene in 70% of P. intermedia-positive samples (Table 11 and Figures 4, 5 and 6). These genes are known to be major pathogenic factors among periodontal bacteria.

Table 11: Distribution of virulence factor genes

Genes	Positive n (%)	Negative n (%)	Total n (%)	p value
fimA	6 (60)	4 (40)	10 (100)	0.046*
adpC	7 (70)	3 (30)	10 (100)	0.0001*

**Significant difference at 0.05 expressed as mean and standard deviation*

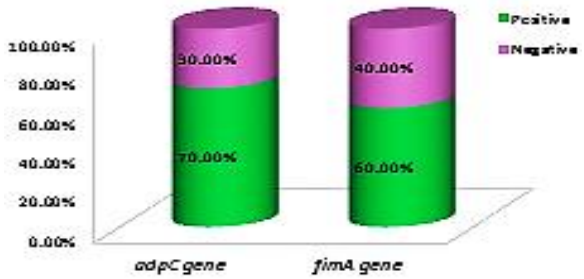

Figure 4: Percentages of virulence genes

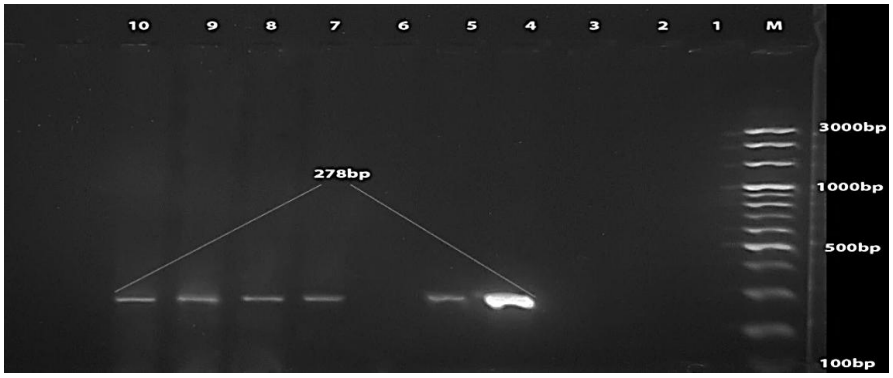

Figure 5: Agarose gel electrophoresis image showing PCR products amplified with fimA-specific primers from ten P. gingivalis isolates.

Lane M: 100 bp DNA ladder (molecular size marker); Lanes 1–10: PCR products of clinical isolates. A 278 bp band confirms the presence of the fimA gene in positive samples. Clear size markers and labelled lanes allow comparison and verification of product size

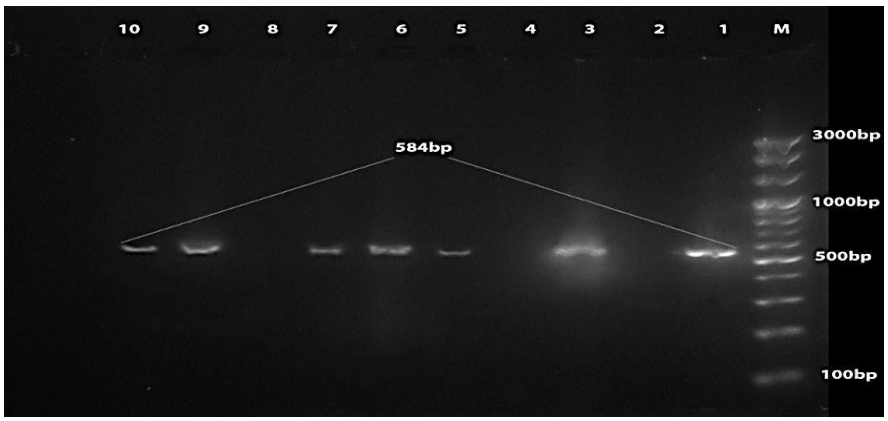

Figure 6: Agarose gel Electrophoresis results of PCR amplification targeting the adpC gene from ten P. intermedia isolates.

Lane M: 100 bp DNA ladder used as a molecular marker. Lanes 1–10: Clinical isolates tested for the adpC gene. Positive samples show a band at 584 bp. Each lane is clearly labelled, and size markers aid in confirming correct amplification

Discussion

The results of the current study concur with those reported by Papone et al., who studied 51 patients diagnosed with chronic periodontitis. Using traditional "microbial" microbiological techniques, these latter authors demonstrated the presence of Aggregatibacter actinomycetemcomitans in all (100%) of these clinical specimens, with black-pigmented anaerobic bacteria. Monoplex PCR analysis revealed that the most commonly prevalent bacteria were P. gingivalis (88%), T. forsythia (92%), and F. nucleatum (100%) (18).

Similar findings were found in the report of Van der Weijden et al., wherein P. intermedia and A. actinomycetemcomitans were the two most frequently detected species, found in 39% and 33% of sites, respectively. Among their study group, P. intermedia, P. gingivalis, and A. actinomycetemcomitans were recovered from 49 (P = 0.0007), 35 (P 0.05). In a similar study (19). Alazemi et al. reported a relatively less isolated P. gingivalis (33.3%) from periodontitis as

compared to other gingival pathogens. In their investigation, which included 30 peri-implant disease patients (14 men and 16 women between 35 and 60 years of age; mean age 45.8 years), they found 26 positive samples—19 for P. intermedia and 7 for P. gingivalis. In the 73 periapical samples examined, 4 (5.48%) were found to be positive for P. intermedia and none for P. gingivalis. Another subset of samples was positive in nine (12.33%) for only P. intermedia, and one sample (1.37%) was found positive for P. gingivalis (20).

In odontogenic stomatitis, P. intermedia and F. nucleatum were commonly found. In Brazilians suffering from periodontal infections, the most frequent species isolated were P. gingivalis (67%), T. forsythia (20%), and A. actinomycetemcomitans (13%) (21). Taba et al. confirmed these results, and P. gingivalis was identified in 26.7% and A. actinomycetemcomitans in 13% of 450 patients with periodontitis (22). In a case-control study performed in patients with oropharyngeal cancer, P. intermedia was detected in 83.3% (20/24), P. gingivalis in 66.7% (16/24), and T. forsythia in 41.7% (10/24) of cases, with significantly higher prevalence compared with the controls (23). The oral cavity hosts an extremely diverse microbiota, and more than 700 bacterial species have been identified to date, thus highlighting the complex microbial ecosystem that inhabits this site (24). Alteration in this equilibrium was related to the initiation of oral diseases, including caries, periodontitis, by and large owing to microbial dysbiosis (25).

While these findings are not in agreement with those of Choi et al., who detected the P. nucleatum to be the most prevalent bacterium in the salivary samples, followed by P. anaerobius, P. intermedia. Values of DNA copies of P. denticola, Campylobacter rectus, Eikenella corrodens, and P. gingivalis were relatively smaller. Such discrepancies may be due to differences in geographic region, genetic background, institutional protocol, personal hygiene, and the technical aspects of sample collection and DNA extraction (26).

Our results are supported by many previous studies that focused on the prevalence of P. gingivalis in patients with periodontitis and gingivitis. Those studies used both non-genomic culture methods and genetic methods (classical polymerase chain reaction (PCR) and real-time PCR to reveal the pathogen. For instance, Boutaga et al. assessed the sensitivity of RT-PCR vs. culture for the detection of P. gingivalis in subgingival plaque. Their findings showed P. gingivalis as 60% and 93.3% positive in group II and group III, respectively. In addition, a participant in group I was also positive for the bacterium (27). Similar results have been reported by Kulkarni et al., who used real-time PCR and culture to study 259 samples obtained from subjects with severe periodontitis. P. gingivalis was detected in 111 (43%) samples by culture and 138 (53%) samples by real-time PCR, confirming the superior sensitivity of molecular assays (28).

Griffen et al. also confirmed these results by analysing 400 chronic periodontitis cases. Their results revealed 73% and 75% P. gingivalis-positive samples by culture and real-time PCR, respectively. For the chronic periodontitis group, detection rates were 89.5% by culture and 91.5% by real-time PCR. By comparison, the detection rates were 54.4% and 58.0% in the healthy control (29). In a study by Kugaji et al., 79.16% (95/120) chronic periodontitis cases were positive for P. gingivalis from the samples in a 120 healthy individual control group (35-29.17%). Our findings are consistent with the study conducted by Joshi et al, showing that 66% of chronic periodontitis patients were positive for P. gingivalis (30). Other studies have recorded even greater levels of prevalence, regularly over 80% (31, 32). P. gingivalis can be identified in healthy individuals but is usually found in lower abundance. This implies that it can transform from a commensal bacterium to a pathogenic organism under certain environmental and/or immunological circumstances (33). Mahdi et al. tested 30 samples of root canals and detected P. intermedia in 6 (20%) samples using molecular methods, and in 10 (33.3%) samples by culture and biochemical analysis (34). In line with this, Milsom et al. demonstrated a 33% detection rate of P. intermedia using PCR versus only a 13% detection rate by culture (35). Additional evidence, from Riggio et al., was demonstrated by the detection of P. intermedia in 38 of 97 subgingival plaque samples from adults through PCR (36). Several studies have reported the existence of remarkably high levels of T. forsythia, P. intermedia, and P. gingivalis in periodontitis patients at some sites, in some cases 20 times greater than those observed in healthy subjects (37).

In a nested case-control study, Castañeda-Corzo et al. demonstrated P. intermedia in 83.3% of PD patients as against 25% in healthy individuals, which was found to be a very significant difference [35].

Recent literature has highlighted the association between P. intermedia and various systemic diseases. It is virulent mainly due to its virulence factors, which include cysteine proteinases, adhesions, and strong biofilm formation. These properties allow the organism to invade host cells, facilitate colonisation by other pathogens, and manipulate host immune-mediated

responses, and are involved in the aetiology of periodontal and systemic diseases (1).

The results of the current study were consistent with previous studies, such as the study conducted by Belcheva et al., who reported on the transmission pattern of C. gingivalis from mothers and fathers (caregivers) to their children. In their study, the presence of bacteria was not significantly different in male and female caregivers (38). In a similar study, Kulkarni et al. evaluated 40 subjects in three groups, 60% of them females. These results were in line with those of a study that did not show a significant difference in the distribution of bacteria between males and females (28).

Kugaji et al. carried out a comparative analysis of the frequency of the presence of P. gingivalis between men and women in the SPCP and R groups. Thirty-six cases (61.01%) among the 59 male patients with periodontitis, and 20 (26.66%) male controls of 75 had positive samples. For women participants, the P. gingivalis detection rate in periodontitis patients was 77.04%, while that in the control group was 33.33%. In the overall population, the bacterium was found in 58.49% of females and 50.74% of males, without a statistically significant difference (30). Similarly, Ingalagi et al. reported that 72% of the periodontitis patients were culture positive for P. gingivalis, and 75% tested positive for P. gingivalis by real-time PCR, without any significant gender difference (39). A study by Abe et al. contextualised this remark by stating that while total bacterial loads were significantly higher in men than in women, the prevalence of pathogens also did not differ strikingly. The findings of this study were explained by hormonal variation and poorer oral hygiene practices in men (40). Choi et al. had also found consistent evidence of F. nucleatum at higher levels in males compared to females, supporting the gender-specific microbial load trend observed in the present study (26).

Tomazinho and Avila carried out a microbiological study of the microbiology of chronic endodontic infections by the bacteriological culture technique and detected P. intermedia in 75.6 % of the PCR-studied samples. No significant differences by gender were identified (41). Another study showed that the co-detection of F. nucleatum and T. forsythia was 100% in male subjects but was significantly lower in females at 83%. The combined detection rate of P. gingivalis, T. forsythia, and F. nucleatum was also higher in men (70%) than in women (39%) (18). Zhang et al. noted that the number of P. intermedia was higher than that of P. gingivalis in subgingival biofilm retrieved from pregnant women during the second and third trimesters. The results of hormonal changes of pregnancy, which are known to irritate existing gingival inflammation (1).

In similar research, Usin et al. Detected P. intermedia in 3.3% of the gingival samples from pregnant women only during the first trimester. The detection of P. intermedia raised the odds of gingival inflammation (GMI > 2) by 51 times (42). In addition, experimental studies concerning gingivitis in pregnancy have recorded an increase in subgingival P. intermedia levels over time.

The findings of the present study were like observations of Kulkarni et al., in which 40 subjects were classified into three groups. The first group had an average age of 29.4±3.80 years, these cases had no infection. The second group had an average of 15 infected cases ‹ 40.60±5.27 years; the third group had an average of infected patients ‹ 45.66±5.08 years. P. gingivalis was detected in 10.00%, in 60.00%, and in 93.33% of non-infected, periodontitis, and chronic periodontitis individuals, respectively (28).

In the same line, Kugaji et al. observed a more substantial rate of P. gingivalis in patients between 41–50 and 51–62 years. Statistically significant difference was detected only between these two age groups, and no statistical difference was observed in younger age groups (21–30, 31–40) (33).

Alazemi et al. reported that P. gingivalis was present in 10 patients of group II and only in 2 healthy controls of the same age group (33.3 vs. 6.4%, respectively) (20). Kareem et al have performed a study in which they crafted grooves in the hyperopic eyes and analysed the frequency of P. gingivalis in COVID-19 patients. The mean age of the infected group and non-infected group was 51.04±13.25 and 47.08±11.45 years, respectively. Age> 40 years was the most affected. Although no statistically significant differences regarding age and gender were observed between the two groups (43).

Griffen et al. observed that subjects with periodontitis were 51.4 years old, and healthy subjects were aged 49.2 years. No significant association was found for detection of P. gingivalis with age; however, for older individuals, a higher detection rate was observed (29). Mättö et al. applied PCR to identify P. gingivalis in subjects 0–18 years old and demonstrated that it was present in 37% of the samples from all age groups, thus indicating that colonisation of the oral cavity can be initiated early in life (44, 45).

In paediatric patients, Tanaka and coworkers associated P. intermedia with halitosis in caries-active as well as caries-free children, showing no difference between the two groups (46). Okada et al. identified P. intermedia in 25% of gingivitis

and periodontitis, affected children aged 2 to 12 years, but found no positives in children without inflammation (47). Lie et al. found that 73% of the 22-year-old subjects were positive for P. intermedia (48).

Alazemi et al. also reported that P. intermedia was detected in 27 (90%) of 50–59-year-old individuals with periodontitis and in 30 (96.7%) age-matched, periodontally healthy subjects [18]. Merchant et al. evaluated adults aged 60 years or older and demonstrated that there was worse cognitive performance when the serum antibody level of IgG against P. intermedia in periodontally infected subjects was increased, revealing that the pathogen has a systemic effect (49). These results are contrary to those of Ambrosio et al., which were performed for 60 participants with an average age of 30 years. They found that P. intermedia was present in most infected sites (97%) while F. nucetatum was less frequently detected (50).

Fukui et al. 13 evaluated bacterial spread among family members and observed the presence of P. intermedia in the three members (mother, father, and son) of 6 families. A total of 293 strains were isolated (number of strains per individual: 5-20). Of the 18 individuals screened, 7 (39%) were identified to be carriers. The ages of the parents were between 36 and 49 years (average age 42.6 years), and the ages of the children ranged from 4 to 15 years (average age 11.0 years) (51). In the current study, there was also an age-related increase in the prevalence of P. intermedia. This finding is in line with the report by Umeda et al., who suggested that the severity of periodontitis is likely to be elevated with age based on cumulative damage to periodontal tissues over time rather than immunological failure due to ageing (52).

Our results are by the study of Rodrigues et al., which analysed several clinical parameters such as the gingival index, plaque index, PD, and BoP. Their study showed a P. gingivalis detection rate in 61.96% of clinical samples and was more prevalent in grade C periodontitis patients. The fimA genotype II type had strong correlations with increased probing depth and percentage of bleeding on probing sites (53). Several other investigations supported the pathogenic role of certain fimA genotypes. In this context, fimA II and IV genotypes by PCR analysis were observed in 3.6% and 0.7% of healthy subjects and 19.6% and 16.5% of periodontitis patients, respectively (54). Shimoyama et al. also reported fimA genotype I in 7.4% and type IV in 37% of apparently healthy children (55).

In another broader study that included 95 patients with periodontitis and 35 healthy individuals, P. gingivalis genotyping isolates showed the highest prevalence of fimA type II among patients with periodontitis (55.89%), whereas fimA type IV was 30.52%. In healthy individuals, however, fimA type I (75.42%) was the predominant type. These results demonstrate a strong association of fimA types II and IV with periodontitis, while fimA type I is strongly associated with periodontal health (56). Krishnan et al. found that fimA type II was present in the chronic and aggressive periodontitis population at a frequency of 50.5% and 45.3%, respectively, versus 13.6% in healthy controls. The prtC gene was detected in a total of 28.9% and 33.3 % of chronic and aggressive periodontitis samples, respectively. In addition, the genetic combination fimA+/prtC+ was detected in 49.5% of the periodontitis patients, and only in 9.1% of the healthy individuals, and it again represents the integrated clinical significance of these genotypes (57).

Van der Ploeg et al. found P. gingivalis in 71% of those with periodontitis and 9% of periodontally healthy subjects. The majority (73%) of positive samples were infected with multiple fimA genotypes. Genotype II was the most common (60%), followed by genotype IV (20%), with genotypes I, III, and V being relatively infrequent (58). Zhao et al. similarly detected that fimA type I was 81.4 % prevalent in periodontally healthy subjects, whereas type II/IV was more highly detected in periodontitis (59).

This trend was similarly reflected in a European population study by Beikler et al.: genotype I was also the most prevalent in healthy individuals (60). In contrast, the work by Missailidis et al. 2 fimA genotype in Brazilian patients and inferred some possible geographic and ethnic variables that could justify the distribution of the genotypes between the populations (61).

For P. intermedia, examination of virulence genes indicated that 70% of the ten isolates harboured the adp gene. Rodriguez Herrero et al. showed that if the bacterial cultures are supplemented with dead P. intermedia or P. gingivalis, adp and estP genes are upregulated by 1.96–4.21-fold in comparison to cultures without addition (62). Sengupta et al. described the gene product of adpF as a leucine-rich repeat domain protein, which mediates bacterial uptake by eukaryotic cells (63). Similarly, Iyer et al. could demonstrate the internalin-like character of this protein in P. intermedia with a similar functional repertoire (64).

Taken together, these virulence factors substantially contribute to its pathotype interference with the host through invasion of host tissues, evasion of the immune response, and the establishment of persistent colonisation, confirming its role in chronic gingivitis or periodontitis (1).

Study limitations
Several limitations should be acknowledged in this study. The relatively small sample size (n = 90) and the fact that participants were recruited from a single centre may restrict the extent to which these findings can be generalised to broader populations. Also, the reliance on PCR-based detection, while sensitive, carries fundamental risks of false negatives, especially due to primer mismatches or limitations in specificity, which are particularly relevant when dealing with genetically diverse strains of P. gingivalis and P. intermedia.

Conclusion
The present investigation revealed again that P. intermedia and P. gingivalis were highly prevalent in gingivitis and periodontitis patients, P. intermedia. Age and sex were not significantly associated with bacterial load, although there was increased bacterial copy number among older subjects. The prevalence of fimA and adpC virulence factors in most of the isolates emphasises their significance in bacterial adherence and immune avoidance. These results indicate a possible role of molecular detection and gene profiling as useful approaches to establish the diagnosis and to monitor therapy in patients with periodontal diseases.
In summary, this investigation emphasises the major prevalence of P. intermedia and P. gingivalis among patients with periodontitis, with important virulence genes such as fimA and adpC frequently identified. The persistent detection of fimA emphasises its likely essential function in biofilm development and tissue invasion, supporting its potential as a promising target for therapeutic intervention in chronic periodontitis management (65). What's more, the observed higher incidence of P. intermedia compared to data from Western populations (66) suggests possible regional variability in oral microbial profiles. This variability emphasises the importance of developing prevention and treatment strategies personalised to specific populations. Future approaches could focus on targeting species-specific virulence mechanisms to effectively disrupt biofilm-related pathogenic processes.
Future research needs to incorporate longitudinal studies that track bacterial load and virulence gene expression before and after periodontal therapy. This approach can clarify the microbial dynamics over time. Also, implementing functional assays, such as gene knockout studies, will be valuable for confirming the specific roles of fimA, adpC, and other virulence factors in biofilm development and tissue invasion.

List of Abbreviations
PCR: Polymerase chain reaction
qPCR: quantitative PCR

Declarations
Ethical consideration and consent to participate
This research received ethical clearance from the Institutional Review Board (IRB) of [University of Al-Qadisiyah], with approval number [IRB/2024/55]. Before sample collection, written informed consent was obtained from all participants or their legal guardians.

Consent for Publication
All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

Availability of Data
Data for this work is available from the authors and may be provided upon reasonable request.

Conflicts of Interest
None.

Funding
None.

Authors' contributions
ARS: Designed the study, supervised clinical sample collection, performed statistical analysis, and contributed to manuscript drafting and critical revision.
MHQ: Conducted laboratory experiments including DNA extraction and real-time PCR, compiled and analysed molecular data, and participated in manuscript writing and figure preparation.

Acknowledgment
The authors are very thankful to the Department of Biology, College of Education, Al Qadisiyah University, for providing the facilities and laboratory assistance throughout this study. Acknowledgements The author thanks the employees of the private dental clinics in Al-Diwaniyah for their cooperation in collecting the samples. The authors would like to thank the members of the Molecular Biology Laboratory team for technical help in the DNA extraction and PCR techniques.

References
1. Zhang S, Zhao Y, Lalsiamthara J, Peng Y, Qi L, Deng S, Wang Q. Current research progress on Prevotella intermedia and associated diseases. Critical Reviews in Microbiology. 2024 Oct 16:1-8.

https://doi.org/10.1080/1040841X.2024.2390594
2. Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, Bei K. Oral microbiome: a review of its impact on oral and systemic health. Microorganisms. 2024 Sep;12(9):1797. https://doi.org/10.3390/microorganisms12091797
3. Wu L, Zhang SQ, Zhao L, Ren ZH, Hu CY. Global, regional, and national burden of periodontitis from 1990 to 2019: Results from the Global Burden of Disease study 2019. Journal of Periodontology. 2022 Oct;93(10):1445-54. https://doi.org/10.1002/JPER.21-0469
4. Casadevall A, Pirofski LA. Virulence factors and their mechanisms of action: the view from a damage–response framework. Journal of water and health. 2009 Aug 1;7(S1):S2-18. https://doi.org/10.2166/wh.2009.036
5. Huang YK, Lee WF, Wang MJ, Chang YH, Tchaou WS, Chang WJ, Lee SY, Sheu JR, Teng NC. Chair-side quantitative oral-microflora screening for assessing familial correlation of periodontal status and caries prevalence. PLoS One. 2014 Jan 30;9(1):e87100. https://doi.org/10.1371/journal.pone.0087100
6. Kirakodu SS, Govindaswami M, Novak MJ, Ebersole JL, Novak KF. Optimizing qPCR for the quantification of periodontal pathogens in a complex plaque biofilm. The open dentistry journal. 2008 Mar 11;2:49. https://doi.org/10.2174/1874210600802010049
7. Decat E, Cosyn J, De Bruyn H, Miremadi R, Saerens B, Van Mechelen E, Vermeulen S, Vaneechoutte M, Deschaght P. Optimization of quantitative polymerase chain reactions for detection and quantification of eight periodontal bacterial pathogens. BMC Research Notes. 2012 Dec;5:1-9. https://doi.org/10.1186/1756-0500-5-664
8. Lopes MP, Cruz ÁA, Xavier MT, Stöcker A, Carvalho-Filho P, Miranda PM, Meyer RJ, Soledade KR, Gomes-Filho IS, Trindade SC. Prevotella intermedia and periodontitis are associated with severe asthma. Journal of Periodontology. 2020 Jan;91(1):46-54. https://doi.org/10.1002/JPER.19-0065
9. Hameed NA, Hasan MS, Mohammed RA, Najim TM. Molecular Detection of Pophyromonas gingivalis and Some Interleukins Related with Periodontal Inflammatory Diseases. Revista iberoamericana de psicología del ejercicio y el deporte. 2022;17(3):75-9.
10. Ruan Y, Shen L, Zou Y, Qi Z, Yin J, Jiang J, Guo L, He L, Chen Z, Tang Z, Qin S. Comparative genome analysis of Prevotella intermedia strain isolated from infected root canal reveals features related to pathogenicity and adaptation. BMC Genomics. 2015 Dec;16:1-21. https://doi.org/10.1186/s12864-015-1272-3
11. How KY, Song KP, Chan KG. Porphyromonas gingivalis: an overview of periodontopathic pathogen below the gum line. Frontiers in microbiology. 2016 Feb 9;7:53. https://doi.org/10.3389/fmicb.2016.00053
12. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. Journal of oral and maxillofacial pathology. 2019 Jan 1;23(1):122-8. https://doi.org/10.4103/jomfp.JOMFP_304_18
13. Abdulkareem AA, Al-Taweel FB, Al-Sharqi AJ, Gul SS, Sha A, Chapple IL. Current concepts in the pathogenesis of periodontitis: from symbiosis to dysbiosis. Journal of Oral Microbiology. 2023 Dec 31;15(1):2197779. https://doi.org/10.1080/20002297.2023.2197779
14. Könönen E, Fteita D, Gursoy UK, Gursoy M. Prevotella species as oral residents and infectious agents with potential impact on systemic conditions. Journal of Oral Microbiology. 2022 Dec 31;14(1):2079814. https://doi.org/10.1080/20002297.2022.2079814
15. Bielecki M, Antonyuk S, Strange RW, Siemińska K, Smalley JW, Mackiewicz P, Śmiga M, Cowan M, Capper MJ, Ślęzak P, Olczak M. Prevotella intermedia produces two proteins homologous to Porphyromonas gingivalis HmuY but with different heme coordination mode. Biochemical Journal. 2020 Jan 31;477(2):381-405. https://doi.org/10.1042/BCJ20190607
16. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nature Reviews Immunology. 2015 Jan;15(1):30-44. https://doi.org/10.1038/nri3785
17. How KY, Song KP, Chan KG. Porphyromonas gingivalis: an overview of periodontopathic pathogen below the gum line. Frontiers in microbiology. 2016 Feb 9;7:53. https://doi.org/10.3389/fmicb.2016.00053
18. Papone V, Verolo C, Zaffaroni L, Batlle A, Capo C, Bueno L, Gamonal J, Silva N, Soria S. Detection and prevalence of periodontal pathogens in a Uruguayan population with chronic periodontitis using conventional methodology and metagenomics. Odontoestomatología. 2015 May;17(25):23-32.

19. Van der Weijden GA, Timmerman MF, Reijerse E, Wolffe GN, Van Winkelhoff AJ, Van der Velden U. The prevalence of A. actinomycetemcomitans, P. gingivalis and P. intermedia in selected subjects with periodontitis. Journal of Clinical Periodontology. 1994 Oct;21(9):583-8. https://doi.org/10.1111/j.1600-051X.1994.tb00747.x
20. Alazemi AM, Jamal W, Al Khabbaz A, Rotimi VO. Prevalence of target anaerobes associated with chronic periodontitis. Access Microbiology. 2020 Nov 16:acmi000177. https://doi.org/10.1099/acmi.0.000177
21. Pfau EA, Avila-Campos MJ. Prevotella intermedia and Porphyromonas gingivalis isolated from osseointegrated dental implants: colonization and antimicrobial susceptibility. Brazilian Journal of Microbiology. 2005;36:281-5. https://doi.org/10.1590/S1517-83822005000300014
22. Taba Jr M, Souza SL, Mariguela VC. Periodontal disease: a genetic perspective. Brazilian oral research. 2012;26:32-8. https://doi.org/10.1590/S1806-83242012000700006
23. Riggio MP, Lennon A, Roy KM. Detection of Prevotella intermedia in subgingival plaque of adult periodontitis patients by polymerase chain reaction. Journal of periodontal research. 1998 Aug;33(6):369-76. https://doi.org/10.1111/j.1600-0765.1998.tb02212.x
24. Chattopadhyay I, Verma M, Panda M. Role of oral microbiome signatures in diagnosis and prognosis of oral cancer. Technology in cancer research & treatment. 2019 Aug 1;18:1533033819867354. https://doi.org/10.1177/1533033819867354
25. Irfan M, Delgado RZ, Frias-Lopez J. The oral microbiome and cancer. Frontiers in immunology. 2020 Oct 23;11:591088. https://doi.org/10.3389/fimmu.2020.591088
26. Choi H, Kim E, Kang J, Kim HJ, Lee JY, Choi J, Joo JY. Real-time PCR quantification of 9 periodontal pathogens in saliva samples from periodontally healthy Korean young adults. Journal of periodontal & implant science. 2018 Aug 30;48(4):261. https://doi.org/10.5051/jpis.2018.48.4.261
27. Boutaga K, van Winkelhoff AJ, Vandenbroucke-Grauls CM, Savelkoul PH. Comparison of real-time PCR and culture for detection of Porphyromonas gingivalis in subgingival plaque samples. Journal of Clinical Microbiology. 2003 Nov;41(11):4950-4. https://doi.org/10.1128/JCM.41.11.4950-4954.2003
28. Kulkarni MR, Bhat KG, Thomas BS, Bhat GS, Kulkarni RD. Identification of multiple strains of Porphyromonas gingivalis using heteroduplex polymerase chain reaction in varying severity of chronic periodontitis. Indian Journal of Medical Microbiology. 2018 Jan 1;36(1):81-6. https://doi.org/10.4103/ijmm.IJMM_17_434
29. Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ. Prevalence of Porphyromonas gingivalis and periodontal health status. Journal of Clinical Microbiology. 1998 Nov 1;36(11):3239-42. https://doi.org/10.1128/JCM.36.11.3239-3242.1998
30. Kugaji MS, Muddapur UM, Bhat KG, Joshi VM, Kumbar VM, Peram MR. Quantitative evaluation of Porphyromonas gingivalis in Indian subjects with chronic periodontitis by Real-Time Polymerase Chain Reaction. Journal of Advanced Oral Research. 2019 Nov;10(2):137-44. https://doi.org/10.1177/2320206819863952
31. Joshi VM, Bhat KG, Kugaji MS, Ingalagi PS. Prevalence of Porphyromonas gingivalis and its relationship with herpesvirus in Indian subjects with chronic periodontitis: A cross-sectional study. Journal of the International Clinical Dental Research Organisation. 2016 Jul 1;8(2):106-10. https://doi.org/10.4103/2231-0754.173791
32. Ingalagi P, Bhat KG, Kulkarni RD, Kotrashetti VS, Kumbar V, Kugaji M. Detection and comparison of prevalence of Porphyromonas gingivalis through culture and Real Time-polymerase chain reaction in subgingival plaque samples of chronic periodontitis and healthy individuals. Journal of Oral and Maxillofacial Pathology. 2022 Apr 1;26(2):288. https://doi.org/10.4103/jomfp.jomfp_163_21
33. Chen LL, Wu YM, Yan J, Sun WL, Sun YZ, David O. Association between coinfection of Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans and Treponema denticola and periodontal tissue destruction in chronic periodontitis. Chinese Medical Journal. 2005;118(11):915-21.
34. Mahdi AA, Al Warid RJ, Al-Ameedee AH. Isolation and Identification of Prevotella Intermedia by Culture and Polymerase Chain Reaction. Indian Journal of Public Health Research & Development. 2019 May 1;10(5). https://doi.org/10.5958/0976-5506.2019.01177.X
35. Milsom SE, Sprague SV, Dymock D, Weightman AJ, Wade WG. Rapid differentiation of Prevotella intermedia and P. nigrescens by 16S rDNA PCR-RFLP. Journal

of Medical Microbiology. 1996 Jan;44(1):41-3. https://doi.org/10.1099/00222615-44-1-41
36. Hyvärinen K, Laitinen S, Paju S, Hakala A, Suominen-Taipale L, Skurnik M, Könönen E, Pussinen PJ. Detection and quantification of five major periodontal pathogens by single copy gene-based real-time PCR. Innate Immunity. 2009 Aug;15(4):195-204. https://doi.org/10.1177/1753425908101920
37. Castañeda-Corzo GJ, Infante-Rodríguez LF, Villamil-Poveda JC, Bustillo J, Cid-Arregui A, García-Robayo DA. Association of Prevotella intermedia with oropharyngeal cancer: A patient-control study. Heliyon. 2023 Mar 1;9(3). https://doi.org/10.1016/j.heliyon.2023.e14293
38. Kobayashi N, Ishihara K, Sugihara N, Kusumoto M, Yakushiji M, Okuda K. Colonization pattern of periodontal bacteria in Japanese children and their mothers. Journal of periodontal research. 2008 Apr;43(2):156-61. https://doi.org/10.1111/j.1600-0765.2007.01005.x
39. Ingalagi P, Bhat KG, Kulkarni RD, Kotrashetti VS, Kumbar V, Kugaji M. Detection and comparison of prevalence of Porphyromonas gingivalis through culture and Real Time-polymerase chain reaction in subgingival plaque samples of chronic periodontitis and healthy individuals. Journal of Oral and Maxillofacial Pathology. 2022 Apr 1;26(2):288. https://doi.org/10.4103/jomfp.jomfp_163_21
40. Abe M, Mitani A, Hoshi K, Yanagimoto S. Large gender gap in oral hygiene behavior and its impact on gingival health in late adolescence. International Journal of Environmental Research and Public Health. 2020 Jun;17(12):4394. https://doi.org/10.3390/ijerph17124394
41. Tomazinho LF, Avila-Campos MJ. Detection of Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia, and Prevotella nigrescens in chronic endodontic infection. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2007 Feb 1;103(2):285-8. https://doi.org/10.1016/j.tripleo.2006.05.010
42. Gil L, Mínguez I, Caffesse R, Llambés F. Periodontal disease in pregnancy: the influence of general factors and inflammatory mediators. Oral Health Prev Dent. 2019 Jan 1;17(1):69-73.
43. Kareem HH, Al-Ghurabi BH, Albadri C. Molecular detection of Porphyromonas gingivalis in COVID-19 Patients. Journal of Baghdad College of Dentistry. 2022 Jun 15;34(2):52-61. https://doi.org/10.26477/jbcd.v34i2.3145
44. Mättö J, Saarela M, Alaluusua S, Oja V, Jousimies-Somer H, Asikainen S. Detection of Porphyromonas gingivalis from saliva by PCR by using a simple sample-processing method. Journal of Clinical Microbiology. 1998 Jan 1;36(1):157-60. https://doi.org/10.1128/JCM.36.1.157-160.1998
45. McClellan DL, Griffen AL, Leys EJ. Age and prevalence of Porphyromonas gingivalis in children. Journal of Clinical Microbiology. 1996 Aug;34(8):2017-9. https://doi.org/10.1128/jcm.34.8.2017-2019.1996
46. Tanaka S, Yoshida M, Murakami Y, Ogiwara T, Shoji M, Kobayashi S, Watanabe S, Machino M, Fujisawa S. The relationship of Prevotella intermedia, Prevotella nigrescens and Prevotella melaninogenica in the supragingival plaque of children, caries and oral malodor. Journal of Clinical Pediatric Dentistry. 2008 Apr 1;32(3):195-200. https://doi.org/10.17796/jcpd.32.3.vp657177815618l1
47. Okada M, Hayashi F, Nagasaka N. PCR detection of 5 putative periodontal pathogens in dental plaque samples from children 2 to 12 years of age. Journal of Clinical Periodontology. 2001 Jun;28(6):576-82. https://doi.org/10.1034/j.1600-051x.2001.028006576.x
48. Lie MA, Van Der Weijden GA, Timmerman MF, Loos BG, Van Steenbergen TJ, Van Der Velden U. Occurrence of Prevotella intermedia and Prevotella nigrescens in relation to gingivitis and gingival health. Journal of Clinical Periodontology. 2001 Feb;28(2):189-93. https://doi.org/10.1034/j.1600-051x.2001.028002189.x
49. Merchant AT, Yi F, Vidanapathirana NP, Lohman M, Zhang J, Newman-Norlund RD, Fridriksson J. Antibodies against periodontal microorganisms and cognition in older adults. JDR Clinical & Translational Research. 2023 Apr;8(2):148-57. https://doi.org/10.1177/23800844211072784
50. Ambrosio N, Marín MJ, Laguna E, Herrera D, Sanz M, Figuero E. Detection and quantification of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans in bacteremia induced by interdental brushing in periodontally healthy and periodontitis patients. Archives of oral biology. 2019 Feb 1;98:213-9. https://doi.org/10.1016/j.archoralbio.2018.11.025
51. Fukui K, Kato N, Kato H, Watanabe K, Tatematsu N. Incidence of Prevotella intermedia and Prevotella nigrescens

carriage among family members with subclinical periodontal disease. Journal of Clinical Microbiology. 1999 Oct 1;37(10):3141-5. https://doi.org/10.1128/JCM.37.10.3141-3145.1999
52. Umeda M, Chen C, Bakker I, Contreras A, Morrison JL, Slots J. Risk indicators for harboring periodontal pathogens. Journal of Periodontology. 1998 Oct;69(10):1111-8. https://doi.org/10.1902/jop.1998.69.10.1111
53. Rodrigues RS, Silveira VR, Rego RO. Analysis of Porphyromonas gingivalis fim A genotypes in severe periodontitis patients. Brazilian Oral Research. 2020 Aug 7;34:e090. https://doi.org/10.1590/1807-3107bor-2020.vol34.0090
54. Amano A, Kuboniwa AM, Nakagawa I, Akiyama S, Morisaki I, Hamada S. Prevalence of specific genotypes of Porphyromonas gingivalis fimA and periodontal health status. Journal of Dental Research. 2000 Sep;79(9):1664-8. https://doi.org/10.1177/00220345000790090501
55. Amano A, Kuboniwa AM, Nakagawa I, Akiyama S, Morisaki I, Hamada S. Prevalence of specific genotypes of Porphyromonas gingivalis fimA and periodontal health status. Journal of dental research. 2000 Sep;79(9):1664-8. https://doi.org/10.1177/00220345000790090501
56. Kugaji M, Muddapur U, Bhat K, Joshi V, Manubolu M, Pathakoti K, Peram MR, Kumbar V. Variation in the Occurrence of fimA Genotypes of Porphyromonas gingivalis in Periodontal Health and Disease. International Journal of Environmental Research and Public Health. 2020 Mar;17(6):1826. https://doi.org/10.3390/ijerph17061826
57. Krishnan M, Krishnan P, Natarajan S. Occurrence of Porphyromonas gingivalis fimA type II and prtC genotype among periodontitis patients. BMC Infectious Diseases. 2012 Jan 1;12(Suppl 1):P22. https://doi.org/10.1186/1471-2334-12-S1-P22
58. van der Ploeg JR, Giertsen E, Lüdin B, Mörgeli C, Zinkernagel AS, Gmür R. Quantitative detection of Porphyromonas gingivalis fimA genotypes in dental plaque. FEMS microbiology letters. 2004 Mar 1;232(1):31-7. https://doi.org/10.1016/S0378-1097(04)00064-3
59. Zhao L, Wu YF, Meng S, Yang H, OuYang YL, Zhou XD. Prevalence of fimA genotypes of Porphyromonas gingivalis and periodontal health status in Chinese adults. Journal of periodontal research. 2007 Dec;42(6):511-7. https://doi.org/10.1111/j.1600-0765.2007.00975.x
60. Beikler T, Peters U, Prajaneh S, Prior K, Ehmke B, Flemmig TF. Prevalence of Porphyromonas gingivalis fimA genotypes in Caucasians. European journal of oral sciences. 2003 Oct;111(5):390-4. https://doi.org/10.1034/j.1600-0722.2003.00065.x
61. Missailidis CG, Umeda JE, Ota-Tsuzuki C, Anzai D, Mayer MP. Distribution of fimA genotypes of Porphyromonas gingivalis in subjects with various periodontal conditions. Oral microbiology and immunology. 2004 Aug;19(4):224-9. https://doi.org/10.1111/j.1399-302X.2004.00140.x
62. Rodriguez Herrero E, Boon N, Pauwels M, Bernaerts K, Slomka V, Quirynen M, Teughels W. Necrotrophic growth of periodontopathogens is a novel virulence factor in oral biofilms. Scientific reports. 2017 Apr 24;7(1):1107. https://doi.org/10.1038/s41598-017-01239-9
63. Sengupta D, Kang DJ, Anaya-Bergman C, Wyant T, Ghosh AK, Miyazaki H, Lewis JP. Interaction of Prevotella intermedia strain 17 leucine-rich repeat domain protein AdpF with eukaryotic cells promotes bacterial internalization. Infection and Immunity. 2014 Jun;82(6):2637-48. https://doi.org/10.1128/IAI.01361-13
64. Iyer D, Anaya-Bergman C, Jones K, Yanamandra S, Sengupta D, Miyazaki H, Lewis JP. AdpC is a Prevotella intermedia 17 leucine-rich repeat internalin-like protein. Infection and immunity. 2010 Jun;78(6):2385-96. https://doi.org/10.1128/IAI.00510-09
65. Alaei SR, Park JH, Walker SG, Thanassi DG. Peptide-based inhibitors of fimbrial biogenesis in Porphyromonas gingivalis. Infection and Immunity. 2019 Mar;87(3):10-128. https://doi.org/10.1128/IAI.00750-18
66. Torrungruang K, Jitpakdeebordin S, Charatkulangkun O, Gleebbua Y. Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Treponema denticola/Prevotella intermedia co-infection are associated with severe periodontitis in a Thai population. PLOS One. 2015 Aug 27;10(8):e0136646. https://doi.org/10.1371/journal.pone.0136646