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Molecular Identification of Porphyromonas gingivalis in Clinical Dentistry

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Abstract

Background: *Porphyromonas gingivalis* is a major periodontal pathogen associated with chronic periodontitis and various systemic diseases. Accurate identification is critically important for diagnosis and treatment decisions. **Objective**: The aims of the present study were to evaluate the molecular techniques for the detection of *P. gingivalis* in dental clinical samples and to compare these methods with conventional culture techniques.
Methods: Subgingival plaque samples were collected from 80 participants with varied severity degree of periodontitis, during 2024 at two dental clinics at Thi-Qar province/Iraq. The samples were cultured and identified using the standard culture procedure, followed by molecular analysis based on polymerase chain reaction (PCR) technique of the 16S rRNA.
Results: PCR testing revealed the prevalence of *P. gingivalis* to be 73.8%, whereas it was detected through culture in only 45%.
Conclusions: The results revealed that PCR is more specific and sensitive than culture for the detection of *P. gingivalis*. Such approaches contribute much for clinical diagnostic in management of treatment of periodontal disease.

Keywords: *Porphyromonas gingivalis, Oral Microbiology, PCR, Clinical Dentistry, Periodontitis.*

Introduction

Periodontal disease is a common chronic inflammatory disease that occurs in populations around the world. About 11% of the globe's adults have severe periodontitis (1). *Porphyromonas gingivalis* is a key pathogen associated with periodontal disease, although it has very low prevalence in the subgingival biofilm (2). *P. gingivalis* is a black-pigmented, Gram-negative, anaerobic bacterium that expresses numerous virulence factors including gingipains, fimbriae, lipopolysaccharides and capsular polysaccharide (3). These factors provide the organism with mechanisms to avoid host immune responses, disturb tissue homeostasis and generate a dysbiotic microbial community that leads to periodontal destruction (4). In addition to its involvement in oral diseases, an increasing number of studies are associating *P. gingivalis* with systemic conditions including cardiovascular disease, rheumatoid arthritis, diabetes mellitus and Alzheimer's disease (5, 6). Routine methods of identification, including culture-dependent techniques, biochemical tests, and microscopic examination have many drawbacks such as prolonged incubation times, strict anaerobic requirement, technical complexity or reduced sensitivity in patients receiving antimicrobial therapy (7). These limitations have stimulated the use of molecular diagnostics that provide fast, accurate and sensitive determination of periodontal pathogens (8).

Molecular methods, especially nucleic acid amplification–based ones, have revolutionized how microbes are detected in clinical settings. Polymerase chain reaction (PCR)-based targeting the species-specific gene sequences allows direct detection of *P. gingivalis* among clinical strains, without cultivation (9). The 16S

rRNA gene is suitable for bacterial identification as it is highly conserved and contains species-specific variations (10). Molecular diagnostics are advantageous in some aspects, but are not commonly used in dental clinics due to the high costs, high technicalities and lack of standardization because they are expensive (11). In order to make good decisions about using these methods and understanding the results, there is need to know how they work in practice. The aim of this study were determination of molecular identification methods for detection the presence of *P. gingivalis* in these clinical dental samples and comparison performance to conventional culture technique. The study seek to furnish evidence advocating for the incorporation of molecular diagnostics into clinical periodontal practice and to enhance comprehension of the epidemiology and pathogenicity of *P. gingivalis* among various patient demographics.

Material and Methods

Study Design and Patients Selection

Eighty patients aged 25-65 years were recruited from two dental clinics at Thi-Qar province/ Iraq during 2024, based on the following criteria: they had at least 20 natural teeth, were diagnosed with gingivitis or chronic periodontitis according to the 2017 World Workshop classification, and had not received antibiotic therapy within three months prior to sample collection. Exclusion criteria included pregnancy or lactation, systemic conditions requiring prophylactic antibiotics, immunosuppressive conditions or medications, current smoking (to eliminate confounding variables), and use of antimicrobial mouthwashes within four weeks of sampling. Patients were categorized into three groups based on periodontal status: healthy/gingivitis (n=20, probing depth ≤3mm with bleeding on probing), moderate periodontitis (n=30, probing depth 4-5mm with clinical attachment loss 3-4mm), and severe periodontitis (n=30, probing depth ≥6mm with clinical attachment loss ≥5mm).

Clinical Examination

Comprehensive periodontal examination was performed by a single calibrated examiner (intra-examiner reliability κ=0.89) using a UNC-15 periodontal probe. Probing pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP) and plaque index (PI) were recorded at six sites around each tooth. The observations were all recorded at the nearest millimeter.

Samples Collection

Subgingival plaque samples were collected from the deepest periodontal pocket in each quadrant (four sites per patient) after removal of supra-gingival plaque with sterile cotton pellets. Sterile paper points (ISO size 30) were inserted into the periodontal pocket for 30 seconds, then immediately placed in separate sterile microcentrifuge tubes containing 500µL of reduced transport fluid (RTF) for culture and 500µL of TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) for molecular analysis.

Conventional Culture and Identification

Culture samples were serially diluted (10^{-1} to 10^{-5}) in pre-reduced phosphate-buffered saline and plated on blood agar supplemented with hemin (5µg/mL), vitamin K1 (1µg/mL), and 5% sheep blood. Plates were incubated at 37°C in anaerobic conditions (85% N2, 10% H2, 5% CO2) for 7-14 days.

DNA Extraction

Samples in TE buffer were vortexed vigorously for 1 minute and paper points removed. Bacterial cells were pelleted by centrifugation at 13000×g for 10 minutes at 4°C. DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol with modifications. Briefly, pellets were resuspended in 180µL enzymatic lysis buffer containing lysozyme (20mg/mL) and mutanolysin (5000U/mL) then incubated at 37°C for 30 minutes. Proteinase K digestion and subsequent steps followed standard protocol. DNA was eluted in 100µL elution buffer and quantified using NanoDrop spectrophotometry. DNA quality was assessed by A260/A280 ratio (acceptable range 1.8-2.0) and stored at -20°C until analysis.

PCR Amplification for Species Identification

Species-specific PCR targeting the 16S rRNA gene was performed using primers designed based on *P. gingivalis*-specific sequences:

Forward primer: 5'-AGGCAGCTTGCCATACTGCG-3'

Reverse primer: 5'-ACTGTTAGCAACTACCGATGT-3'

Expected amplicon size: 404 bp

PCR amplifications were performed in 25 µl reaction volume, including: 12.5µL GoTaq Green Master Mix (Promega, USA), 1µL forward and reverse primer (10pmol/ul), 2µL template DNA (~50 ng), and 8.5µL nuclease-free water. Amplification was carried out in a PCR (Bio-Rad T100) with the protocols of 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, and an appropriate annealing step (60°C) for 45 sec and extension at 72°C for 45 sec, and final extension at 72°C for 10 min.

Statistical Analysis

Statistical analysis was performed using SPSS version 26.0 (IBM Corp., USA). Homogeneity of variances was checked with the Levene test and normality using the Shapiro-Wilk method. Quantitative variables were described as mean ± SD or median with interquartile range, when necessary. Categorical variables were reported as numbers and percentages. To determine the predictive factors of the outcome, univariate and multivariate logistic regression analyses were performed. The logistic regression analysis results were presented as odds ratios (OR) and their 95% confidence intervals (CI). P< 0.05 was regarded as statistical significance.

Result

Study Population Characteristics

A total of 80 patients with a mean age of 42.6 ± 11.3 years (38 males, [47.5%] and 42 females [52.5%]) were enrolled in the study. The proportion across periodontal status categories was: healthy/gingivitis (n=20, 25%), moderate periodontitis (n=30, 37.5%), and severe periodontitis (n=30, 37.5%). Mean clinical parameters for the entire cohort were: PPD 4.8 ± 2.1mm, CAL 4.2 ± 2.4mm, BOP 58.3 ± 24.7%, and PI 1.8 ± 0.6 (Table 1).

Table 1. Study Population Characteristics (N=80).

Demographics Characteristic		**Value**
Age (years), Mean ± SD		42.6 ± 11.3
Sex, n (%)	Male	38 (47.5%)
	Female	42 (52.5%)
Periodontal Status, n (%)	Healthy / Gingivitis	20 (25.0%)
	Moderate Periodontitis	30 (37.5%)
	Severe Periodontitis	30 (37.5%)
Clinical Parameters, Mean ± SD	Probing Pocket Depth (PPD, mm)	4.8 ± 2.1
	Clinical Attachment Level (CAL, mm)	4.2 ± 2.4
	Bleeding on Probing (BOP, %)	58.3 ± 24.7
	Plaque Index (PI)	1.8 ± 0.6

Detection of P. gingivalis: Culture versus PCR

Conventional culture methods detected *P. gingivalis* in 36 of 80 patients (45.0%), while PCR-based molecular identification detected the organism in 59 patients (73.8%) (Figure 1). The difference between detection methods was statistically significant (*P*<0.001). Among the 59 PCR-positive samples, 33 were also culture-positive (55.9%), while 26 samples (44.1%) were culture-negative but PCR-positive. All culture-positive samples were confirmed positive by PCR, with three culture-positive samples showing negative PCR results upon initial testing but positive upon repeat analysis, suggesting potential inhibition or technical issues (Table 2).

Table 2. Comparison of *P. gingivalis* Detection Methods

Method	No. of Positive Patients	Percentage	P-value
Culture	36	45.0%	<0.001
PCR	59	73.8%	

The sensitivity of PCR relative to culture (considered as combined positive) was 93.9%, with specificity of 87.8%. Cohen's kappa coefficient indicated substantial agreement between methods (κ=0.72, 95% CI: 0.58-0.86, P<0.001) (Table 3).

Table 3. Agreement between Culture and PCR Methods

Metric	Value	Interpretation / *P*-value
Sensitivity of PCR	93.9%	-
Specificity of PCR	87.8%	-
Cohen's Kappa (κ)	0.72	Substantial Agreement
95% CI for κ	0.58 - 0.86	-
P-value for κ	<0.001	Statistically significant agreement

Detection rates varied significantly across periodontal status groups. In the healthy/gingivitis group, *P. gingivalis* was detected by culture in 15% (3/20) and by PCR in 30% (6/20). In moderate periodontitis, detection rates were 46.7% (14/30) by culture and 76.7% (23/30) by PCR. In severe periodontitis, rates were 63.3% (19/30) by culture and 100% (30/30) by PCR. The trend toward increased detection with disease severity was significant for both methods (*P*<0.001) (Table 4).

Table 4. *P. gingivalis* Detection by Periodontal Status

Periodontal Status	Culture Positive	PCR Positive	*P*-value
Healthy / Gingivitis (n=20)	3 (15.0%)	6 (30.0%)	<0.001
Moderate Periodontitis (n=30)	14 (46.7%)	23 (76.7%)	
Severe Periodontitis (n=30)	19 (63.3%)	30 (100.0%)	

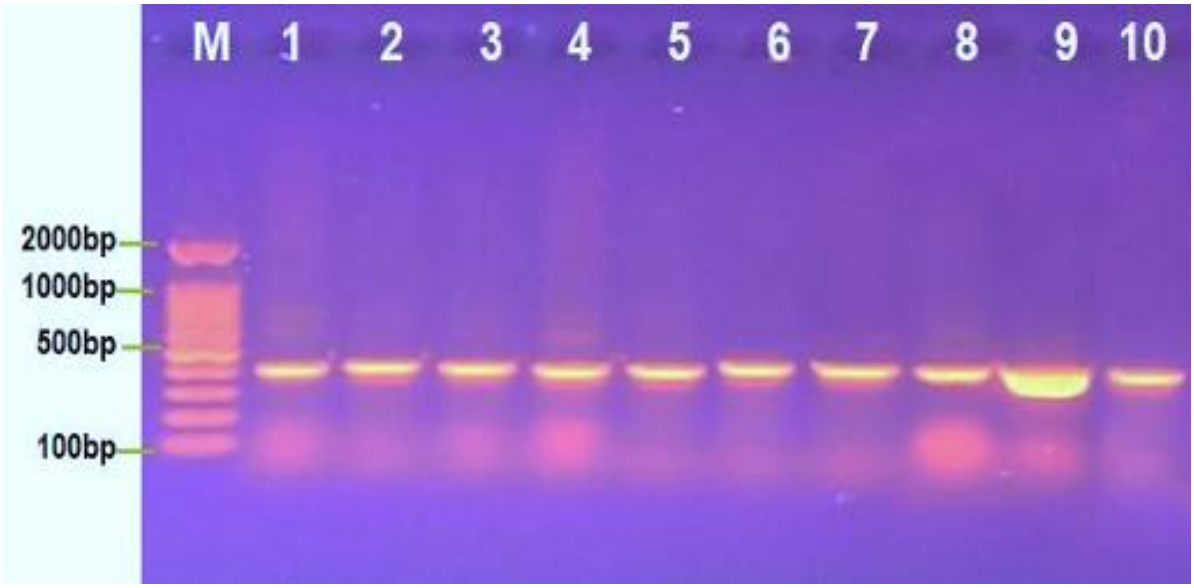

Figure 1. Agarose gel electrophoresis image that show the PCR product analysis of 16S rRNA gene. Where M: marker (2000-100bp), lane (1-10) show some positive *P. gingivalis* species at (404 bp) PCR product size.

Predictors of P. gingivalis Detection

Multiple logistic regression analysis was performed to identify independent predictors of *P. gingivalis* detection via PCR. The model revealed that a diagnosis of severe periodontitis was the strongest significant predictor (Adjusted OR = 12.4, 95% CI: 3.2-48.1, *P*<0.001). Furthermore, the presence of deep periodontal pockets (PPD ≥6 mm; Adjusted OR = 8.7, 95% CI: 2.4-31.5, *P*=0.001) and significant clinical attachment loss (CAL ≥5 mm; Adjusted OR = 6.3, 95% CI: 1.9-20.8, *P*=0.002) were also strong and statistically significant independent predictors. Patient age over 45 years was a weaker but still significant predictor (Adjusted OR = 2.8, 95% CI: 1.1-7.4, *P*=0.035). In contrast, gender, plaque index, and bleeding on probing did not demonstrate a statistically significant independent association with *P. gingivalis* detection in the adjusted model (Table 5).

Table 5. Predictor Variables of *P. gingivalis* Detection by PCR

Predictor Variable	**Odds Ratio (OR)**	**95% Confidence Interval (CI)**	***P*-value**
Significant Predictors			
Severe Periodontitis (vs. Healthy/Gingivitis)	12.4	3.2 - 48.1	<0.001***
Probing Pocket Depth ≥ 6 mm	8.7	2.4 - 31.5	0.001**
Clinical Attachment Loss ≥ 5 mm	6.3	1.9 - 20.8	0.002**
Age > 45 years	2.8	1.1 - 7.4	0.035*
Non-Significant Predictors			
Gender	NS	-	>0.05
Plaque Index	NS	-	>0.05
Bleeding on Probing	NS	-	>0.05

Discussion

This study demonstrates that molecular methods, especially PCR-based techniques are more sensitive and have greater clinical relevance than the traditional culture for detection of *P. gingivalis* in periodontal samples. The 73.8% detection rate by PCR in comparison with a 45.0% by culture demonstrates significant enhancement of diagnostic capacity, which is consistent with other studies that shows sensitivity differences that have ranged from 1.5 to 2.5 fold over for molecular methods in comparison to culture methods (8, 13). The enhanced sensitivity of molecular techniques is partly due to numerous reasons. PCR detects bacterial DNA directly and is not dependent on the viability or cultivability of the bacteria, a feature that facilitates diagnosis of living but nonculturable (VBNC) bacteria, which can remain present in biofilms, or may be an effect of prior antimicrobial induction (14). All the while, *P. gingivalis* has stringent anaerobic requirements and picky growth characteristics that make it difficult to culture. The transport of the sample and processing may compromise the viability of the organism even with reduced transport fluid (7). The molecular approach also eliminates the subjective interpretation of colony morphology and biochemical tests (9). The presence of *P. gingivalis* in 30% of healthy/gingivitis individuals as detected by PCR raises important questions regarding the role of the organism in maintaining oral health and causing disease. This finding supports the keystone pathogen hypothesis that *P. gingivalis*, is capable of dysbiotic changes in the microbial community leading to disease, even among what appear to be healthy sites (2, 4). The lack of type V evidenced in the Nagasaki study population is noteworthy, although not unique, as this genotype has been known to display geographic variation with high frequency amongst Japanese and low among Western populations (15). Detection of type-specific infection could be influenced by the efficiency of primer design and amplification competition in mixed infections (10). Severe periodontitis, deepened periodontal pockets, extensive attachment loss and age above 45 years were independent variables multiple logistic analysis significant as predictors of *P. gingivalis* infection. These results align with epidemiology of periodontal disease and could inform risk-stratified sampling strategies (16). The absence of gender differences in detection rates, while consistent with some studies, contrasts with reports suggesting hormonal influences on subgingival bacterial composition and thus necessitates further research (17). Clinically, the diagnostic time for molecular identification of 6.5 hours is in distinct contrast to that >10 days by culture and would have an important impact on timely diagnosis and planning (11). Although traditional culture might still be useful for antimicrobial susceptibility testing, molecular assays allow us to identify rapidly the pathogens that can guide empirical therapy selection, particularly for adjunctive systemic antibiotics in severe periodontitis management (18). The cost effectiveness of molecular tests is still a consideration for its routine clinical use. Initial equipment investment and per specimen reagent costs are higher than those for culture, but automation, multiplexing capacity and reduced labor requirements offer potential advantages in cost optimization at high volume for molecular assays (19). The clinical benefits of improved and earlier diagnostic precision should be balanced with economic considerations, where cost-effectiveness evaluations would be necessary for decision-making about adoption (20). Several limitations should be acknowledged. The cross-sectional nature of the survey prevents assessment of any stability in genotype colonization or association between molecular findings and longitudinal treatment response. The lack of smokers, while eliminating an important confounding variable, limits generalizability to this high-risk population (21). Sampling four sites per patient has been standardized, but may not reflect the total microbial composition at all periodontal sites (22). Furthermore, molecular techniques identify both viable and non-viable organisms, which may lead to an overestimation of active infection in comparison with culture of viable organisms (14). Future research directions include longitudinal studies to evaluate genotype stability and alterations following periodontal therapy, identification of molecular markers for antibiotic resistance as an aid in antimicrobial selection, correlation of genotype-specific infections with their systemic disease associations, development of chairside molecular diagnostic devices for point of care testing, and economic analyses to determine cost effectiveness of molecular diagnostics integrated into periodontal practice protocols (11).

The incorporation of molecular microbiology into clinical periodontology signifies a transformative shift towards precision medicine methodologies. Genotype-specific information might eventually determine individualized

treatment strategies, with high-virulence genotypes possibly necessitating more intensive therapeutic measures (12).

Conclusion

This study demonstrates that PCR is significantly more sensitive for the detection of *P. gingivalis* from periodontal samples than conventional culture methods with detection rate is 73.8% compared to 45.0%. Molecular methods also offered fast results in 6.5 hours, which makes it easier to make timely clinical decisions. *P. gingivalis* was found in 30% of healthy individuals, supporting the hypothesis that keystone pathogens precipitate dysbiosis. Culture methods still have useful, but molecular diagnostics are better for finding the main pathogen. The shift to those technologies in clinical practice would involve consideration of the technical infrastructure and costs.

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Conflict of interest

There is no confluent of interests for the author.

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