

## Characterization of *Porphyromonas gingivalis* in Chronic Oral Inflammatory Conditions Using Molecular Techniques in Eastern India

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### ABSTRACT

*Porphyromonas gingivalis* is a primary periodontal pathogen associated with chronic oral inflammatory conditions such as periodontitis and gingivitis. Its presence and virulence in oral biofilms play crucial roles in tissue damage and the systemic inflammatory load. This study identified *P. gingivalis* in patients with chronic oral inflammation at the Kalinga Institute of Dental Sciences, Bhubaneswar using molecular diagnostic methods. This study involved 60 patients aged 25–60 years who were diagnosed with chronic periodontitis or severe gingivitis. Subgingival plaque samples were collected under sterile conditions, cultured, and examined for black pigmented colonies. Biochemical characterization and DNA extraction were performed using established techniques. *P. gingivalis* was detected by polymerase chain reaction (PCR) targeting the 16S rRNA gene, followed by gel electrophoresis. Positive samples were sequenced, compared with known strains, and submitted to the NCBI database. *P. gingivalis* was identified in 42 out of 60 patients (70%). Patients with severe periodontitis had higher bacterial loads than those with mild-to-moderate inflammation. Sequencing confirmed the pathogenic characteristics of these isolates. This study highlights the role of molecular diagnostics in identifying periodontal pathogens in Eastern India, thereby supporting accurate diagnosis and treatment. These findings provide molecular epidemiological insights into Odisha and improve our understanding of oral microbial diversity in Indian populations.

**Keywords:** Chronic periodontitis, Molecular diagnostics, 16S rRNA PCR, Oral microbiome, Gingival inflammation, Periodontal pathogens

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### 1. INTRODUCTION

Chronic inflammatory diseases affecting the mouth, such as chronic periodontitis and severe gingivitis, represent considerable public health challenges in Eastern India, especially in Odisha [1]. These ailments are mainly caused by dysbiotic subgingival biofilms, with *Porphyromonas gingivalis* identified as a key pathogen in their development. *P.*

*gingivalis* is a Gram-negative, anaerobic bacterium that produces virulence factors like gingipains, fimbriae, capsules, and lipopolysaccharides (LPS), which aid in evading the immune system, damaging host tissues, and sustaining the disease [2,3].

Epidemiological research indicates a significant positive link between the presence of *P. gingivalis* and the severity of periodontal disease, with detection rates increasing markedly with increasing probing pocket depth (PPD) and clinical attachment loss (CAL) [5]. For example, PCR-based methods have shown prevalence rates of about 60% in pockets measuring  $\leq 5$  mm and over 90% in areas deeper than 5 mm [3,6]. Similarly, a recent study found detection rates of up to 93% in deep pockets ( $> 5$  mm) compared to approximately 60% in shallower sites [7].

Conventional culture-based techniques are still laborious and may overlook strains that are either low in abundance or are difficult to cultivate. In contrast, real-time PCR and isothermal amplification methods, such as RPA or LAMP, provide quick and sensitive alternatives for detection and quantification [8]. In India, comparative research has demonstrated that RT-PCR identifies *P. gingivalis* in almost 90% of chronic periodontitis cases, compared to approximately 55% in healthy individuals, both of which are significantly higher than results from culture methods alone [8]. Additionally, new point-of-care devices have been evaluated, showing a strong correlation ( $r \approx 0.73$ ) with PCR outcomes and periodontal indicators, such as PPD and CAL [9].

Molecular and phylogenetic analyses have further demonstrated that *P. gingivalis* possesses an open pangenome, with virulence factors such as fimbriin (FimA), Mfa1, and hemagglutinins being conserved among different strains, aiding host colonization and contributing to pathogenic diversity [10]. Emerging strain-specific associations indicate that virulent lineages, such as W83, are associated with more severe disease phenotypes across various populations [11].

Although it is a significant global pathogen, there is a lack of region-specific data from Eastern India, especially Odisha. Factors such as local oral hygiene practices, microbial ecology, and host immune responses may affect the prevalence and distribution of bacterial strains in this area. It is essential to establish epidemiological and molecular baselines, particularly in tertiary care facilities such as the Kalinga Institute of Dental Sciences in Bhubaneswar. Gaining insights into the local prevalence and genetic characteristics of *P. gingivalis* can guide targeted periodontal treatment, antimicrobial management, and potential screening methods.

This experimental study was conducted in a laboratory setting using molecular diagnostics, specifically 16S rRNA PCR and sequencing, to analyze *P. gingivalis* in subgingival plaque samples from individuals with chronic periodontitis and severe gingivitis at the Kalinga Institute of Dental Sciences, Bhubaneswar. This study aimed to (i) assess prevalence rates among symptomatic patients, (ii) examine the relationship between bacterial load and clinical parameters such as PPD, CAL, and GI, and (iii) conduct phylogenetic analysis to place local strains within the context of global lineages. Hospitals in low- to middle-income areas, such as Odisha, could benefit from rapid and precise diagnostics to enable early intervention.

## 2. MATERIALS AND METHODS

### Study Design and Population

This cross-sectional study was conducted from January to June 2025 in the Department of Periodontology, Kalinga Institute of Dental Sciences, Bhubaneswar, Odisha. This study included 60 participants aged between 25 and 60 years who were clinically diagnosed with chronic periodontitis or severe gingivitis. Diagnoses were based on the 2018 guidelines of the American Academy of Periodontology (AAP)/EFP, which specify probing pocket depth (PPD  $\geq 5$  mm), clinical attachment loss (CAL  $\geq 3$  mm), and bleeding on probing (BOP). Individuals with systemic diseases, those who had taken antibiotics in the previous three months, or those who had received periodontal treatment in the past six months were excluded. Before the study commenced, approval was obtained from the Kalinga Institute of Dental Sciences Institutional Ethical Committee (reference No: KIIT/KIMS/IEC/1807/2024), and all participants provided written informed consent.

### Clinical Examination

A single calibrated examiner used a UNC-15 periodontal probe to document periodontal parameters such as probing pocket depth (PPD), clinical attachment level (CAL), plaque index (PI), and gingival index (GI) with the aim of reducing variability between observers.

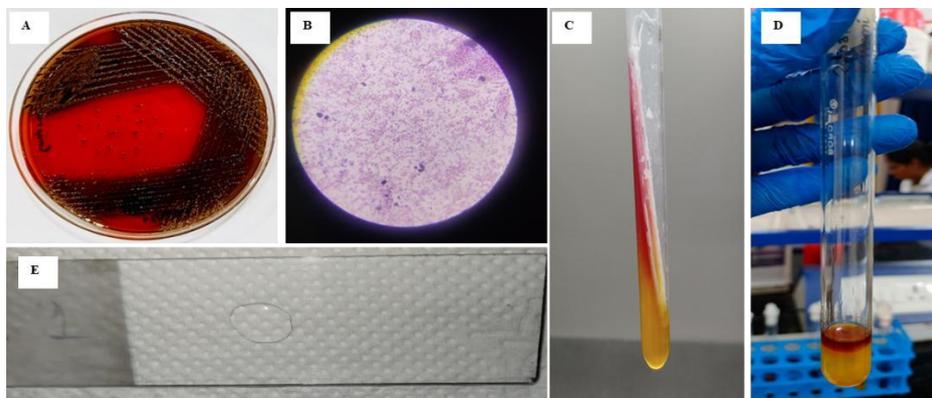
### Sample Collection

Subgingival plaque samples were obtained from the deepest periodontal pocket of each patient by using sterile Gracey curettes. These samples were then placed in sterile Eppendorf tubes containing TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

### Isolation of *P. gingivalis* from Plaque Samples via Anaerobic Culture

Pooled plaque samples were serially diluted in phosphate-buffered saline (PBS) and then cultured on Blood Agar plates enriched with sterile human serum and Vitamin K using the pour plate method. The plates were incubated in an anaerobic

environment for 7–8 d. Black-pigmented colonies were subcultured (Figure-1) and subjected to Gram staining. Colonies identified as Gram-negative coccobacilli were subjected to biochemical testing. Isolates that tested positive for indole, negative for catalase, and showed sugar fermentation were tentatively identified as *P. gingivalis* and were chosen for DNA extraction and further molecular analysis.



**Figure 1:** Isolation and identification of *Porphyromonas gingivalis* from pooled plaque samples. (A) Blood agar plates enriched with human serum and vitamin K and incubated anaerobically for 7–8 days, showing characteristic black-pigmented colonies. (B) Gram staining of Gram-negative bacilli. (C) Triple Sugar Iron (TSI) agar test results. (D) Positive indole test results indicating tryptophanase activity. (E) Catalase test results confirming biochemical characteristics.

### DNA Extraction

Genomic DNA was extracted using the Qiagen bacterial DNA extraction kit (Qiagen, Germany) according to the manufacturer's protocol, with slight modifications to the biofilm samples. The DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific).

### PCR Amplification of *P. gingivalis*

*P. gingivalis* was detected using species-specific primers targeting the conserved region of the 16S rRNA gene to ensure high specificity and sensitivity. The primer sequences used were: Forward primer 27F, 5'-AGAGTTTGATCCTGGCTCAG-3' and Reverse primer 1429R; 5'-GGTTACCTTGTTACGACTT-3'). The polymerase chain reaction (PCR) was set up in a total reaction volume of 25  $\mu$ L, comprising 12.5  $\mu$ L of 2X Taq PCR Master Mix, 0.5  $\mu$ M of each primer, 2  $\mu$ L of extracted template DNA, and nuclease-free water to make up the volume. Amplification was carried out using a thermocycler (Takara Bio, Shiga, Japan) under the following conditions: initial denaturation at 95  $^{\circ}$ C for 5 min, followed by 35 cycles of denaturation at 95  $^{\circ}$ C for 30 s, annealing at 60  $^{\circ}$ C for 30 s, and extension at 72  $^{\circ}$ C for 1 min. The final elongation step was carried out at 72  $^{\circ}$ C for 5 min. The amplified PCR products were separated on a 1.5% agarose gel stained with a green fluorescent dye (Barcoad Biosciences, India). The bands were then visualized under UV transillumination (Biorad, USA), indicating the presence of *P. gingivalis*.

### Sequencing of *Porphyromonas gingivalis* Isolates

PCR-positive amplicons were purified using a PCR cleanup kit from Genestoprotein (India) before sequencing. To assess genetic similarity with *P. gingivalis* reference strains, multiple sequence alignments were performed using MEGA11. The sequences were analyzed using NCBI BLAST for confirmation and were submitted to the NCBI database. The Accession number is PX067033.

### Statistical Analysis

Descriptive statistics were used to determine the prevalence of *P. gingivalis* infection. The relationships between the clinical parameters (PPD and CAL) and the presence of bacteria were examined using chi-square tests and independent t-tests. Statistical significance was set at  $P < 0.05$ . Data analysis was performed with SPSS version 26.0.

## 3. RESULTS

### Demographic and Clinical Characteristics

Sixty patients with chronic oral inflammatory conditions were included in the study. The mean age of the participants was  $42.6 \pm 9.2$  years, with 38 males (63.3%) and 22 females (36.7%). Clinically, 44 patients (73.3%) were diagnosed with chronic periodontitis, whereas 16 (26.7%) had severe gingivitis.

### Culture-Based Detection of *P. gingivalis*

Of the 60 pooled subgingival plaque samples cultured on Blood Agar under anaerobic conditions, 42 samples (70%) showed growth of small, round, black-pigmented colonies, with or without hemolysis, after 7–8 days of incubation. Gram staining confirmed the presence of Gram-negative coccobacilli in 26 of these 42 isolates (Table-1).

Analysis of the clinical indices showed significant links between *P. gingivalis* and periodontal disease severity. *P. gingivalis*-positive patients had higher probing pocket depth (PPD:  $6.1 \pm 1.2$  mm) versus negatives ( $4.5 \pm 0.9$  mm;  $p < 0.001$ ). Clinical attachment loss was greater in positives ( $4.8 \pm 1.0$  mm) than negatives ( $3.2 \pm 0.8$  mm;  $p < 0.01$ ). Gingival index values were higher in *P. gingivalis*-positive patients ( $2.6 \pm 0.4$ ) than in negatives ( $2.1 \pm 0.5$ ;  $p < 0.05$ ). Plaque index showed no significant difference between groups ( $2.4 \pm 0.6$  vs.  $2.2 \pm 0.7$ ;  $p > 0.05$ ), indicating *P. gingivalis* relates more to disease severity than plaque levels (Table-2).

Further biochemical characterization revealed that 18 of the 26 gram-negative isolates were positive for indole, negative for catalase, and non-fermentative in sugar tests, which is consistent with the typical characteristics of *P. gingivalis*. These 18 culture-confirmed isolates were selected for DNA extraction and further molecular confirmation using 16S rRNA gene PCR.

#### Association with Clinical Parameters

Patients with *P. gingivalis*-positive cultures exhibited significantly deeper periodontal pockets, greater clinical attachment loss, and higher gingival index scores than those with negative cultures. No statistically significant differences were observed in plaque index values between the two groups.

**Table 1: Demographic Distribution and *P. gingivalis* Detection**

Parameter	n (%)	<i>P. gingivalis</i> Positive (n = 42)	<i>P. gingivalis</i> Negative (n = 18)
Total Patients	60 (100%)	42 (70%)	18 (30%)
Male	38 (63.3%)	28 (66.7%)	10 (55.6%)
Female	22 (36.7%)	14 (33.3%)	8 (44.4%)
Mean Age (years)	$42.6 \pm 9.2$	$43.1 \pm 8.5$	$41.3 \pm 9.8$

**Table 2: Association Between Clinical Indices and *P. gingivalis* Detection**

Clinical Parameter	<i>P. gingivalis</i> Positive (n = 42)	<i>P. gingivalis</i> Negative (n = 18)	p-value
Probing Pocket Depth (PPD, mm)	$6.1 \pm 1.2$	$4.5 \pm 0.9$	$< 0.001$
Clinical Attachment Loss (CAL, mm)	$4.8 \pm 1.0$	$3.2 \pm 0.8$	$< 0.01$
Gingival Index (GI)	$2.6 \pm 0.4$	$2.1 \pm 0.5$	$< 0.05$
Plaque Index (PI)	$2.4 \pm 0.6$	$2.2 \pm 0.7$	NS ( $p > 0.05$ )

#### Molecular Confirmation of *Porphyromonas gingivalis* Isolates

Sequencing was conducted on the PCR-positive isolates (n = 10) to confirm their identity. The sequences showed a similarity range of 98–100% based on NCBI BLAST analysis. Using MEGA11 software for multiple sequence alignment, a significant level of conservation was noted in the 16S rRNA gene region, confirming the specificity of the amplification. Phylogenetic analysis placed all isolates within the *P. gingivalis* clade, indicating no deviation from the global pathogenic strains. These results validated the molecular detection protocol and highlighted the pathogenic importance of isolates from the Eastern Indian population.

#### 4. DISCUSSION

In a study conducted at the Kalinga Institute of Dental Sciences in Bhubaneswar involving 60 patients with chronic oral inflammation, *P. gingivalis* was identified in 70% of the cases using species-specific PCR. This detection rate was consistent with that reported in both Indian and international studies. Previous research in India has shown a prevalence of 60–90% among patients with chronic periodontitis, supporting the regional consistency of these findings [12,13].

Patients positive for *P. gingivalis* exhibited significantly greater probing pocket depth (PPD) and clinical attachment loss (CAL) compared to PCR-negative individuals (mean PPD 6.1 mm vs. 4.5 mm; CAL 4.8 mm vs. 3.2 mm;  $p < 0.01$ ). These associations mirror earlier observations linking the *P. gingivalis* microbial load to periodontal disease severity [13]. The Elevated Gingival Index (GI) also correlated with positivity, although plaque index differences were not significant, suggesting that the host inflammatory response may modulate periodontal tissue breakdown beyond mere biofilm formation.

The high prevalence in our cohort supports the keystone-pathogen role of *P. gingivalis* in the subgingival “red complex,” contributing to dysbiosis and chronic inflammation despite being a relatively low-abundance species [14]. The absence of detection by conventional culture methods in many cases underscores the advantage of molecular diagnostics, such as PCR or qPCR, in achieving diagnostic sensitivity, particularly for fastidious anaerobes [15,16]. From a regional perspective, this is among the few molecular epidemiological studies conducted in Odisha. Oral hygiene behaviors, socioeconomic factors, and underlying immunological profiles may influence microbial patterns. Thus, our data provides essential baseline information for local clinical practice and public health strategies.

Limitations include the cross-sectional design, which precludes causal inferences and lacks comparisons with healthy controls. Future research should incorporate quantitative PCR or immunochromatographic assays to estimate bacterial load, longitudinal monitoring post-treatment, and multicenter sampling to increase generalizability. Multivariate analysis, including systemic inflammation markers and host genotypes (e.g., GST or PPARG polymorphisms) and co-infection profiles, would enhance the understanding of host–microbe interactions [17,18].

Clinically, these findings support the implementation of molecular diagnostics as part of periodontal assessment protocols. Identifying *P. gingivalis* in deep pockets can guide targeted antimicrobial therapy, adjunctive interventions (e.g., systemic antibiotics, local antiseptics), and early interventions to prevent progression.

Lastly, the broader significance of *P. gingivalis* beyond oral health continues to emerge; its virulence factors have been implicated in systemic conditions, including cardiovascular disease, insulin resistance, rheumatoid arthritis, and neurodegenerative diseases, suggesting that early detection and control of periodontal infection may yield systemic health benefits [19,20].

## 5. CONCLUSION

This study showed a high prevalence of *Porphyromonas gingivalis* in patients with chronic periodontitis and gingivitis, demonstrating its role in periodontal diseases in Eastern India. The correlation between *P. gingivalis* and clinical indices reinforces its value in clinical assessment. Molecular techniques, particularly 16S rRNA polymerase chain reaction (PCR) and sequencing, detect this pathogen better than traditional methods. Phylogenetic analysis showed homology with globally virulent strains, indicating pathogenic potential. These findings provide data on microbial diversity and disease burden in Odisha. Molecular diagnostics may enhance detection. Intervention against *P. gingivalis* benefits oral health and may reduce the systemic impact. However, further longitudinal studies are required for personalized management. This study supports precision periodontology in low-resource settings.

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All authors are contributed equally

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No

### Conflict of Interest

The authors declare no conflicts of interest.

### Ethics Approval

Yes

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