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Porphyromonas gingivalis prevalence and *gyrA* gene detection linked to mercury exposure in periodontitis among gold miners

HELENA JELITA[1], HARUN ACHMAD[2], SYAMSUL ARIFIN[3], DAHLIA HERAWATI[4], IRENE EDITH RIEUWPASSA[5], RASMIDAR SAMAD[6], ANDI ROFIAN SULTAN[7], HANASIA[8], FIRDAUS HAMID[7,♥]

[1]Department of Dentistry, Faculty of Medicine, Universitas Palangka Raya. Jl. Yos Sudarso, Palangka Raya 74874, Central Kalimantan, Indonesia
[2]Department of Pedodontics, Faculty of Dentistry, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia
[3]Department of Administration and Health Policy, Faculty of Medicine, Universitas Lambung Mangkurat. Jl. Veteran Sungai Bilu No. 128, Banjarmasin 70122, South Kalimantan, Indonesia
[4]Department of Periodontics, Faculty of Dentistry, Universitas Gadjah Mada. Jl. Bulaksumur, Sleman 55281, Yogyakarta, Indonesia
[5]Department of Oral Biology, Faculty of Dentistry, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia
[6]Department of Dental Public Health, Faculty of Dentistry, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia
[7]Department of Microbiology, Faculty of Medicine, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia. Tel.: +62-411-586200, Fax.: +62-411-584200, ♥email: firdaus.hamid@gmail.com
[8]Department of Medical Laboratory Technology, Faculty of Medicine, Universitas Palangka Raya. Jl. Yos Sudarso, Palangka Raya 74874, Central Kalimantan, Indonesia

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Abstract. *Jelita H, Achmad H, Arifin S, Herawati D, Rieuwpassa IE, Samad R, Sultan AR, Hanasia, Hamid F. 2026.* Porphyromonas gingivalis *prevalence and* gyrA *gene detection linked to mercury exposure in periodontitis among gold miners. Biodiversitas 27 (4): d270437. https://doi.org/10.13057/biodiv/d270437*. The oral cavity is a dynamic ecosystem hosting diverse anaerobic microorganisms that influence oral health. *Porphyromonas gingivalis*, a key Gram-negative anaerobe, is strongly implicated in periodontitis pathogenesis. Chronic mercury (Hg) exposure, particularly among artisanal gold miners, has been associated with microbial dysbiosis and antibiotic resistance genes. This study investigated the relationship between salivary Hg levels, subgingival anaerobic bacteria, detection of *P. gingivalis* and *gyrA* in periodontitis among gold miners. A cross-sectional design included 60 participants (30 gold miners and 30 non-gold miners). Subgingival plaque was cultured anaerobically and identified using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS); Polymerase Chain Reaction (PCR) detected *P. gingivalis* and *gyrA*. Salivary Hg concentrations were determined using Atomic Absorption Spectrophotometry with a Mercury Vapor Unit (AAS-MVU). Median salivary Hg levels were significantly higher in miners than non-miners (0.09 µg/L [0.03-0.22] vs. 0.03 µg/L [0.03-0.03]; $p<0.001$), although all values remained below the 5 µg/L salivary threshold. Culture recovery was low, identifying *Prevotella denticola* and *P. melaninogenica* among miners and *Bifidobacterium* sp. among non-miners. PCR detected *P. gingivalis* in 80.00% of miners and 63.33% of non-miners ($p = 0.152$). In contrast, *gyrA* detection was higher in miners (19/30, 63.33%) than in non-miners (10/30, 33.33%, $p = 0.020$). Mann–Whitney analysis showed no significant differences between salivary Hg and detection of *P. gingivalis* ($p = 0.420$) or *gyrA* ($p = 0.953$). However, a significant association between *P. gingivalis* and *gyrA* was observed using the Chi-square test ($\chi^2 = 22.191$, $p<0.001$; $\varphi = 0.61$). Although *P. gingivalis* prevalence did not differ significantly, chronic low-dose Hg exposure was associated with higher *gyrA* detection and distinct anaerobic bacteria in miners, suggesting Hg may be associated with resistance-related microbial dynamics in periodontal biofilms. Larger studies using quantitative PCR and metagenomic approaches are needed to clarify underlying mechanisms.

Keywords: Antibiotic resistance genes, mercury exposure, microbial dysbiosis, periodontal pathogens

INTRODUCTION

Periodontitis is a chronic inflammatory disease characterized by progressive destruction of the gingiva, periodontal ligament, and alveolar bone (Fiorillo et al. 2019). The condition arises from subgingival microbiota dysbiosis, in which an imbalance between commensal and pathogenic microorganisms triggers a destructive host immune response (Saliem et al. 2022). The interface between dental plaque and gingival tissue provides an anaerobic environment supporting pathogenic bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, collectively known as the "red complex", which plays a central role in periodontal tissue destruction (Tian et al. 2024).

Globally, periodontal disease affects nearly half of the global population and remains a major oral health burden (WHO 2023). Clinically, periodontitis presents with periodontal pocket formation (≥5 mm), clinical attachment loss, and alveolar bone resorption (Tao et al. 2025). As a major cause of tooth loss affecting oral function and quality of life, periodontitis is also linked to systemic disorders such as diabetes, cardiovascular disease, and Alzheimer's (Fiorillo et al. 2019; Gabiec et al. 2022).

Among periodontal pathogens, *P. gingivalis* is recognized as a keystone species capable of disrupting host-microbial

homeostasis even at low abundance (Jakubovics et al. 2021). Through immune modulation and biofilm restructuring, it promotes chronic inflammation and ecological imbalance within subgingival communities. The oral microbiome is therefore a dynamic ecosystem highly sensitive to environmental stressors, including heavy metals that may alter microbial composition and tolerant or pathogenic taxa (Davis et al. 2020).

Mercury exposure is especially relevant in Artisanal and Small-Scale Gold Mining (ASGM), where elemental mercury is used for gold amalgamation. Inhalation of mercury vapor, together with indirect exposure through contaminated tools, soil, and water, poses unique occupational risks for miners (Baek et al. 2016; Charkiewicz et al. 2025). Mercury can accumulate in saliva and soft tissues, leading to excessive generation of Reactive Oxygen Species (ROS), oxidative stress, redox imbalance, and DNA damage (Naser et al. 2024). These biochemical alterations may compromise epithelial integrity, impair immune regulation, and disrupt microbial community stability conditions that can favor the proliferation of anaerobic pathogens, including *P. gingivalis* (Zhao et al. 2020).

Epidemiological evidence suggests a potential link between mercury exposure and periodontal disease. Han et al. (2009) reported a positive association between hair mercury levels and periodontitis prevalence. Beyond clinical associations, heavy metals can co-select for antimicrobial resistance through shared stress-response pathways and mobile genetic elements (Conrads et al. 2021). Mercury-induced oxidative stress generates selective pressure within complex biofilms, potentially favoring resistant bacterial variants. In polymicrobial periodontal biofilms, selective pressures may alter community structure and promote bacteria carrying resistance genes, linking environmental metal exposure with antimicrobial resistance in the oral microbiome (Hooper and Jacoby 2015; Pal et al. 2017). One relevant molecular target is DNA gyrase, encoded by the *gyrA* gene, where mutations are associated with fluoroquinolone resistance in advanced or refractory periodontal infections (Ardila and Bedoya-García 2022). Although direct evidence linking mercury exposure to specific *gyrA* mutations remains limited, *gyrA* detection serves as a clinically relevant proxy for resistance-associated ecological selection under metal-induced stress (Kumar et al. 2025).

ASGM communities represent a unique human model of chronic low-dose mercury exposure. Unlike controlled industrial settings, ASGM populations experience continuous environmental exposure through inhalation, dermal contact, and contaminated water sources, generating sustained ecological pressure on resident microbiota (Basu et al. 2015). Periodontitis patients constitute an appropriate clinical model in this context, as periodontal biofilms are dense polymicrobial ecosystems in which environmental stressors may influence microbial shifts and resistance gene persistence (Curtis et al. 2020).

Despite growing recognition of these interactions, empirical data linking mercury exposure to the prevalence of *P. gingivalis* and resistance-associated genes such as *gyrA* in subgingival microbiota remain limited, particularly in ASGM communities. Addressing these gaps, this study aimed to compare salivary mercury levels, subgingival anaerobic bacterial profiles, and the detection of *P. gingivalis* and the *gyrA* gene between periodontitis patients from gold-mining and non-mining populations. We hypothesized that gold miners would exhibit higher salivary mercury levels, distinct anaerobic bacterial profiles, and increased detection of both *P. gingivalis* and the quinolone-resistance-associated *gyrA* gene compared to non-miners. The findings are expected to enhance understanding of how environmental pollutants shape oral microbial dynamics and underscore the need for integrated public health strategies to address mercury-related oral and systemic health risks.

MATERIALS AND METHODS

Study design and participants

This analytical observational study used a comparative cross-sectional design. A total of sixty adult patients with clinically diagnosed chronic periodontitis (30 artisanal gold miners and 30 non-gold miners) were recruited using purposive sampling from community-dwelling populations from three villages in Central Kalimantan, Indonesia: Tewah Village (Tewah Sub-district), Hurung Village (Banama Tingang Sub-district), and Bolukan Village (Bukit Rawi Sub-district). Participants were selected based on predefined inclusion criteria. All eligible participants who met the inclusion criteria and agreed to participate were enrolled in the study. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Hasanuddin (No: 590/UN4.6.4.5.31/PP36/2024). Written informed consent was obtained from all participants. The sample size for this study was determined based on the Lemeshow formula for estimating proportions:

$$n = \frac{\frac{Z_{1-\alpha}^2}{2} \times P \times (1 - P)}{d^2}$$

The sample size was calculated based on a 95% confidence level (Z = 1.96), an assumed conservative Prevalence (P) of 0.5, and a margin of error (d) of 0.20. The use of P = 0.5 represents a conservative estimate that maximizes the required sample size when the true prevalence is unknown. The margin of error of 0.20 was selected due to the exploratory nature of the study and the limited population of eligible miners in the research area. Based on these parameters, the minimum calculated sample size was 24 participants. To account for potential non-participation or sample-related issues, the sample size was increased by 20%, resulting in 30 participants per group (gold miners and non-gold miners), giving a total of 60 participants.

Inclusion and exclusion criteria

Inclusion criteria

(i) Age 18-65 years. (ii) Diagnosed chronic periodontitis, defined as the presence of least site with Probing Pocket Depth (PPD) ≥5 mm or generalized pocketing >4 mm with Clinical Attachment Loss (CAL) consistent with periodontitis

diagnostic criteria. (iii) For gold miners: ≥1 year history of active work in artisanal gold mining involving mercury use. (iv) For non-gold miners: no occupational exposure to mercury or mining activities.

Exclusion criteria

(i) Systemic diseases that may affect periodontal status or immune function (e.g., uncontrolled diabetes mellitus, autoimmune disease, HIV). (ii) Antibiotic or systemic corticosteroid use within the last 3 months. (iii) Current pregnancy or lactation. (iv) Professional periodontal therapy (scaling and root planning) within 3 months prior to sampling. (v) Use of antiseptic mouthwash within 48 hours before sampling.

These conditions were excluded because systemic diseases such as uncontrolled diabetes mellitus, autoimmune disorders, and HIV infection are well known to alter host immune responses, inflammatory regulation, and oral microbiota composition, which could introduce substantial confounding when assessing the relationship between mercury exposure and periodontal bacterial profiles. Similarly, recent antibiotic or corticosteroid use can suppress bacterial load or disrupt subgingival microbial ecology, while pregnancy and lactation involve hormonal fluctuations that may influence periodontal status. Professional periodontal therapy and antiseptic mouthwash use were excluded to avoid alterations in bacterial abundance that could interfere with both culture-based and molecular analyses.

Sample collection

Trained and standardized examiners conducted clinical examinations and sample collection to ensure procedural consistency. Participants were instructed to brush their teeth, abstain from eating, drinking (except water), and smoking for at least one hour prior to sampling. In addition, participants were advised not to consume water during the last 15 minutes before the sample was collected to prevent dilution or alteration of the sample. Unstimulated whole saliva was collected first by the passive drool method, in which participants were asked to allow saliva to naturally pool in the mouth and drool into a sterile tube for 2-3 minutes (approximately 2 mL). Samples were immediately placed on ice and subsequently stored at -20°C until mercury analysis using Atomic Absorption Spectrophotometry with a Mercury Vapor Unit (AAS-MVU). Subgingival plaque samples were collected using aseptic technique from two periodontal pockets with the deepest probing depths in each participant. One sterile paper point (ISO size 60) was inserted into each site for 10 seconds to allow absorption of subgingival plaque. The two paper points obtained from the two different sites were combined (pooled) into a single sterile microtube containing 1 mL of sterile thioglycolate transport medium, resulting in one composite sample per participant. Plaque samples from the two periodontal sites with the deepest probing depths were pooled to obtain a representative composite sample of active disease-associated subgingival microbiota and to ensure sufficient biomass for both culture and molecular analyses. Site-specific variability was not evaluated, as the primary objective was to assess resistance-associated gene detection at the patient level rather than site-level ecological differences. Care was taken to gently dry and isolate the sites with cotton rolls before sampling to prevent salivary contamination. All plaque samples were transported on ice and processed within 2-4 hours for culture-based analysis, while aliquots intended for molecular analysis were stored at -80°C until DNA extraction.

Mercury concentration analysis

Salivary mercury concentrations were determined using Atomic Absorption Spectrophotometry with Mercury Vapor Unit (AAS-MVU) at the Laboratory for Testing the Quality of Medicinal, Food, and Cosmetic Products, Faculty of Pharmacy, Universitas Indonesia. Sample preparation was conducted by transferring 1 mL of saliva into a conical flask and adding bromine monochloride (BrCl) to oxidize all mercury species to Hg^{2+}. The oxidized mercury was subsequently reduced to elemental mercury (Hg^0) using stannous chloride ($SnCl_2$). Quality control procedures included procedural blanks to assess potential reagent and instrument contamination, as well as calibration verification using certified mercury standards. Spike-recovery tests were applied in each analytical batch to evaluate the accuracy of the method. Method validation covered linearity, assessed using standard solutions ranging from 0.05 to 1.0 µg/mL for calibration curve construction. The Limit of Detection (LOD), obtained from repeated reagent-blank measurements, was 0.06 µg/L for salivary mercury. Samples with concentrations below the LOD were reported as "<LOD (0.06 µg/L)" or not detected. Final mercury concentrations were expressed in micrograms per liter (µg/L).

Bacterial isolation and identification

The bacterial isolation procedure followed the guidelines of Srivastava et al. (2020) with modifications. Samples transported in thioglycolate medium were cultured in Brucella agar with hemin and vitamin K1 and supplemented with 5% sheep blood. Inoculated plates were placed in anaerobic jars and incubated at 37°C under an atmosphere consisting of 80% N_2, 10% H_2, and 10% CO_2, generated using GasPack CO_2 sachets. Cultures were maintained for 7-14 days to allow optimal growth of slow-growing anaerobic bacteria. The cultures were examined daily, and all morphologically distinct black-pigmented colonies defined by their dark pigmentation, smooth convex morphology, and absence or presence of hemolysis were selected as representative isolates for further identification. The *P. gingivalis* ATCC 33277 strain was included as a positive control.

Pure cultures of black-pigmented bacterial isolates were analyzed using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) with the VITEK® MS-DS Target Slide system. A bacterial suspension was prepared by emulsifying single colonies in the manufacturer-recommended buffer. Subsequently, 1 µL of the suspension was spotted onto the designated target plate position and allowed to air-dry. An additional 1 µL of α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution was then applied and dried to form matrix-protein co-crystals. The prepared target slide was loaded into the VITEK® MS instrument for spectral acquisition. During

analysis, MALDI-TOF MS generated protein mass spectra through laser desorption/ionization, predominantly detecting ribosomal protein peaks that serve as conserved biomarkers for anaerobic bacteria. The resulting spectra were automatically matched against the VITEK® MS reference database, including validated entries for *P. gingivalis*. Final identification was determined based on the highest-scoring spectrum match and its associated confidence value, enabling reliable differentiation between *P. gingivalis* and other black-pigmented anaerobic species.

DNA extraction

Bacterial DNA extraction was performed using the QIAamp DNA Mini Kit (QIAGEN, Germany; Cat. No. 51304) according to the manufacturer's instructions. A bacterial suspension was prepared in thioglycolate transport medium with a McFarland density of 0.5-1 in an Eppendorf tube. The suspension was then centrifuged for 5 minutes at 300 × g to pellet the cells, and the supernatant was discarded. The pellet was resuspended in 200 µL of Phosphate-Buffered Saline (PBS) for washing the cells. Thereafter, 20 µL of Proteinase K was added to digest proteins. The mixture was then vortexed until homogenized. The mixture was then incubated at 60°C for 20 minutes with intermittent mixing every 5 minutes. Thereafter, 200 µL of ethanol was added, and the mixture was vortexed for 10 seconds to facilitate DNA binding.

The mixture was transferred to a GS column in a 2 mL collection tube and centrifuged at 14,000-16,000 rpm for 1 minute. The resulting liquid was discarded, and the sample was washed with 400 µL of W1 buffer and centrifuged for 30 seconds. The washing procedure was repeated using 600 µL of wash buffer, followed by centrifugation for 30 seconds. The collection tube was then replaced and centrifuged again for 3 minutes to remove residual buffer. The GS column was transferred to a sterile Eppendorf tube, and 100 µL of preheated elution buffer was added. The column was centrifuged at 14,000-16,000 rpm for 30 seconds to elute the DNA. Afterward, the GS column was discarded. The eluate was collected and used as the DNA template for PCR analysis.

Polymerase chain reaction analysis

Detection of *P. gingivalis* was performed using the PCR method with primers previously reported by Murakami et al. (2023). The primer sequences were forward 5′-ACAGAGGGGGATAACCCGTT-3′ and reverse 5′-ATGCAATACTCGTATCGCC-3′, yielding an amplicon of 338 bp. Amplification of the gyrA gene was conducted using primers designed by Rieuwpassa and Hatta (2009): forward 5′-TGATCGTCTCCAGAGCTTTG-3′ and reverse 5′-CCTTATCTATGTCCTGAAGC-3′, generating a PCR product of 339 bp. The PCR reaction composition consisted of 12.5 µL GoTaq® Green Master Mix (Promega, USA; Cat. No M7122), 1 µL Primer (F), 1 µL Primer (R), and 0.5 µL Nuclease-Free Water, with a total reagent mix volume of 15 µL, then a 3 µL of DNA template was added, bringing the total PCR reaction volume to 18 µL. The PCR amplification was conducted using a 2720 Thermal Cycler (Applied Biosystems) under the following conditions, as presented in Table 1. PCR amplicons were separated via 2.0% UltraPure™ agarose gel electrophoresis (Invitrogen, USA; Cat. No. 15581044) in 1X TBE buffer and stained with ethidium bromide for visualization. A 100 bp DNA ladder (Promega, USA; Cat. No. G2101) was utilized as a molecular size reference.

Each PCR run included a positive control (*Escherichia coli* ATCC 25922), a no-template negative control, and reagent blanks to monitor potential contamination. Amplicon specificity was initially verified by agarose gel electrophoresis based on the expected product size and subsequently confirmed by Sanger sequencing of representative positive samples. Only clearly distinguishable bands corresponding to the expected amplicon size were considered positive. Samples exhibiting faint or ambiguous bands were interpreted conservatively and classified as negative to prevent overestimation of detection rates. The detection threshold was defined as the minimum band intensity clearly distinguishable from background fluorescence under standardized UV illumination conditions.

Sequencing

The *gyrA* gene fragments amplified from *P. gingivalis*-positive samples were purified using a PCR purification kit (QIAquick PCR Purification Kit, Qiagen, Germany). Representative positive amplicons were then subjected to Sanger sequencing using a Genetic Analyzer (Applied Biosystems, USA) to confirm target identity. The resulting chromatograms were edited and assembled using BioEdit Sequence Alignment Editor (version 5.0.9). The consensus sequences were compared with reference sequences available in the NCBI GenBank database using the BLASTn algorithm (Basic Local Alignment Search Tool). Sequences displaying ≥97% nucleotide identity to *P. gingivalis gyrA* reference sequences were considered as species-level matches. The primary analysis was based on the presence or absence of gene detection.

Table 1. PCR cycling conditions for amplification of *Porphyromonas gingivalis* and *gyrA* genes

Primer	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
Porphyromonas gingivalis	95°C (2 min)	95°C (30 sec)	60°C (45 sec)	72°C (30 sec)	72°C (5 min)	35
gyrA	94°C (1 min)	94°C (30 sec)	59°C (1.5 min)	72°C (2 min)	72°C (5 min)	34

Data analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 16.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables, including Hg concentration (Hg), PPD, and CAL, was assessed using the Shapiro-Wilk test. The results indicated that the data were not normally distributed ($p<0.05$). Therefore, comparisons of mercury levels, CAL, and PPD between the two independent groups were conducted using the Mann-Whitney U test. Categorical variables, such as the presence of *P. gingivalis* and the *gyrA* gene, were compared between groups using the Chi-square test to assess differences in proportions. To evaluate the relationship between Hg concentration and bacterial detection status, mercury levels were compared between presence and absence groups using the Mann-Whitney U test. Associations between categorical variables, including the co-detection of *P. gingivalis* and *gyrA*, were analyzed using the Chi-square test. All tests were two-tailed, and a p-value of <0.05 was considered statistically significant. Non-parametric tests were selected due to the non-normal distribution of continuous variables.

RESULTS AND DISCUSSION

Demographic profile of study participants

A total of 60 patients diagnosed with chronic periodontitis (30 gold miners and 30 non-gold miners) were enrolled in this study. The demographic distribution of the participants is presented in Table 2.

Among the 60 participants, the distribution of gender and age differed notably between groups (Table 2). Gold miners were predominantly older males, whereas non-gold miners were mainly younger females. The higher proportion of males among gold miners may reflect occupational norms in which men primarily conduct mining activities. Conversely, the predominance of females among non-gold miners may be influenced by differences in occupational exposure and lifestyle factors. Table 2 shows a notable imbalance in both gender and age between the two groups, with gold miners being predominantly older males and non-miners being mostly younger females. Such demographic disparities represent important potential confounders that may influence the microbial and molecular differences observed. Because the present study did not statistically adjust for these variables, the resulting microbial and molecular comparisons should be interpreted with considerable caution.

Individual salivary mercury measurements are provided in while summary statistics are presented in Table 3. Although the mercury levels in miners were significantly higher than non-miners, all measured concentrations remained below the established salivary threshold of 5 µg/L used in dental amalgam exposure guidelines. Therefore, the ecological effects observed likely reflect chronic low-level exposure rather than overt toxic concentrations.

The clinical parameters (CAL and PPD) and mean Hg concentrations for both study groups are shown in Table 3. No significant differences were observed in CAL ($U = 438.5$, $Z = -0.216$, $p = 0.829$) or PPD ($U = 397.0$, $Z = -0.975$, $p = 0.330$) between gold miners and non-gold miners. In contrast, median salivary Hg was higher in miners (0.09 µg/L [IQR 0.03-0.22]) than non-miners (0.03 µg/L [IQR 0.03-0.03]), with a highly significant difference ($U = 250.5$, $Z = -3.372$, $p = 0.001$). These findings indicate that the periodontal clinical status of both groups was relatively similar, whereas mercury exposure differed markedly.

Identification of anaerobic bacteria by MALDI-TOF mass spectrometry

Anaerobic bacterial isolates displayed characteristic black-pigmented colony morphology, which was identified using MALDI-TOF mass spectrometry (Table 4). Overall, culture-based identification yielded very low recovery rate of anaerobic bacteria (<15% across groups), reflecting known methodological limitations of anaerobic cultivation for fastidious subgingival species. No *P. gingivalis* isolates were obtained from either study group. In contrast, PCR detected *P. gingivalis* in a substantially higher proportion of participants with 80.00% in gold miners and 63.33% in non-gold miners (Table 5). The marked difference between culture and PCR outcomes reflects the higher analytical sensitivity of molecular detection, which can identify bacterial DNA even when viable cells are present in low abundance or cannot be recovered through culture.

Table 2. Demographic characteristics of study participants

Characteristic	Gold miners (n = 30)	Non-gold miners (n = 30)
Gender		
Males	23 (76.67%)	8 (26.67%)
Females	7 (23.33%)	22 (73.33%)
Age group		
25-44 years	12 (40%)	23 (76.67%)
45-59 years	18 (60%)	7 (23.33%)
Age (mean±SD)	40.93±7.03 years	

Table 3. Clinical parameters (CAL, PPD) and Hg concentrations in gold miners and non-gold miners

Parameters	Gold miners (n = 30)	Non-gold miners (n = 30)	U	Z	p-value
CAL (mean±SD)	5.83±0.53	5.77±0.50	438.5	-0.216	0.829
PPD (mean±SD)	5.80±0.55	5.93±0.52	397.0	-0.975	0.330
Mercury (µg/L), median (IQR)	0.09 (0.03-0.22)	0.03 (0.03-0.03)	250.5	-3.372	0.001

Note: Mann-Whitney U test was used for group comparisons. Data are presented as mean±SD or median (IQR) as appropriate

Table 4. Anaerobic bacteria identified by MALDI-TOF MS in the study groups

Bacteria	Gold miners (n = 30)	Non-gold miners (n = 30)
Bifidobacterium sp.	0	1 (3.33%)
Prevotella denticola	2 (6.67%)	0
Prevotella melaninogenica	1 (3.33%)	0
Total	3	1

Note: The overall culture recovery rate (<15%) likely reflects methodological limitations of anaerobic cultivation rather than the absence of subgingival bacteria

Table 5. PCR-based detection of *Porphyromonas gingivalis* among study groups

Groups	*Porphyromonas gingivalis* (%)		p-value
	Positive	Negative	
Gold miners	24 (80.00)	6 (20.00)	0.152
Non-Gold miners	19 (63.33)	11 (36.67)	
Total	43 (71.65)	17 (28.35)	

Detection of *Porphyromonas gingivalis* by PCR

Table 5 summarizes the prevalence of *P. gingivalis* detected by PCR in both study groups. The prevalence was higher among gold miners than non-gold miners; however, the difference was not statistically significant ($p = 0.152$). This non-significant finding may be related to the moderate sample size, which limits the statistical power to detect small to medium differences. Representative PCR amplification results for the *P. gingivalis*-specific gene (338 bp) are shown in Figure 1.

Detection of the *gyrA* gene by PCR

Table 6 presents the detection of the *gyrA* gene among the study groups. A higher prevalence was observed in gold miners compared to non-gold miners, and this difference was statistically significant ($p = 0.02$). Given the higher mercury exposure among gold miners, this finding may suggest a possible association between mercury exposure and the presence of *gyrA*. However, causal relationships cannot be established based on the present data and require further investigation. Representative electrophoresis results demonstrating the *gyrA* amplicon (339 bp) are shown in Figure 2.

Escherichia coli ATCC 25922 was used as a negative control to verify the specificity of the primers targeting the *gyrA* gene of *P. gingivalis*. Although *E. coli* also possesses a housekeeping *gyrA* gene, its nucleotide sequence differs significantly from that of *P. gingivalis*, allowing confirmation that no cross-reactivity or non-specific amplification occurred under the established PCR conditions.

Table 7 presents the statistical analysis of the relationship between Hg concentration and bacterial detection, as well as the association between *P. gingivalis* and the *gyrA* gene. No significant differences in Hg levels were observed between subjects with and without *P. gingivalis* ($p = 0.420$; $r = 0.10$) or *gyr*A ($p = 0.953$; $r = 0.01$), indicating negligible effect sizes. In contrast, a statistically significant and strong association was found between *P. gingivalis* and *gyrA* detection ($\chi^2 = 22.191$, $p<0.001$; $\varphi = 0.61$).

Discussion

This study investigated the relationship between salivary mercury exposure, anaerobic bacterial composition, the prevalence of *P. gingivalis*, and the detection of the *gyrA* gene in patients with chronic periodontitis from two groups with distinct environmental exposure characteristics, namely gold miners and non-miners. Hg concentrations were significantly higher among gold miners (median 0.09 µg/L; IQR 0.03-0.22) than among non-miners (median 0.03 µg/L; IQR 0.03-0.03; $p<0.001$), although all values remained below the commonly referenced salivary safety threshold of 5 µg/L. PCR analysis revealed that *P. gingivalis* was detected in 80.00% of gold miners and 63.33% of non-miners. In contrast, culture-based methods yielded very limited recovery of anaerobic bacteria (<15%) and failed to isolate *P. gingivalis*. This may be explained by the strict anaerobic and fastidious growth requirements of *P. gingivalis*, which make it difficult to culture, particularly under suboptimal handling and environmental conditions. Furthermore, the *gyrA* gene was detected significantly more frequently among gold miners (63.33%) than among non-miners (33.33%; $p = 0.02$). Correlation analysis showed no significant association between salivary mercury levels and the presence of either *P. gingivalis* or the *gyrA* gene ($p>0.05$). However, a strong positive correlation between *P. gingivalis* and *gyrA* was observed in both miners ($r = 0.657$) and non-miners ($r = 0.538$). Together, these findings suggest that while environmental mercury exposure may contribute to ecological pressures within the oral microbiome, the observed microbial patterns are likely influenced by multiple biological and demographic factors.

Demographic characteristics differed substantially between the study groups. The gold-miner group was predominantly male (76.67%), whereas the non-miner group consisted mostly of females (73.33%). This distribution reflects occupational patterns typically observed in artisanal and small-scale gold mining, where men primarily undertake mining activities. Such gender imbalance may introduce variability, as sex is biologically known to influence periodontal status, host immune responses, and the composition of the oral microbiome (Su et al. 2023). Previous studies have reported that men generally exhibit greater susceptibility to periodontal tissue destruction and a higher prevalence of pathogenic anaerobic bacteria, potentially due to hormonal differences, systemic inflammatory profiles, smoking habits, or oral health behaviors (Alfitory et al. 2024). Consequently, the predominance of older males among miners and younger females among non-miners may partly contribute to differences in microbial distribution observed in this study.

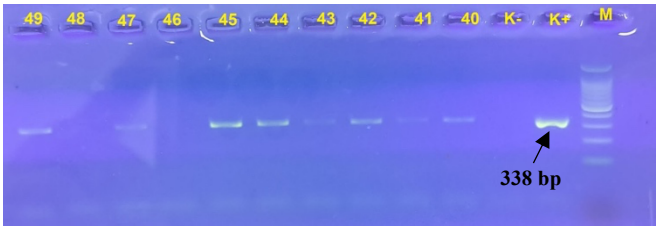

Figure 1. Agarose gel electrophoresis (2.0%) performed in 2023 showing PCR amplification of the *Porphyromonas gingivalis*-specific gene (338 bp), Lane M: 100 bp DNA ladder (100-1500 bp), Lanes 40-49: Clinical samples, K^+: Positive control (*Porphyromonas gingivalis* ATCC 33277), K^-: No-template negative control

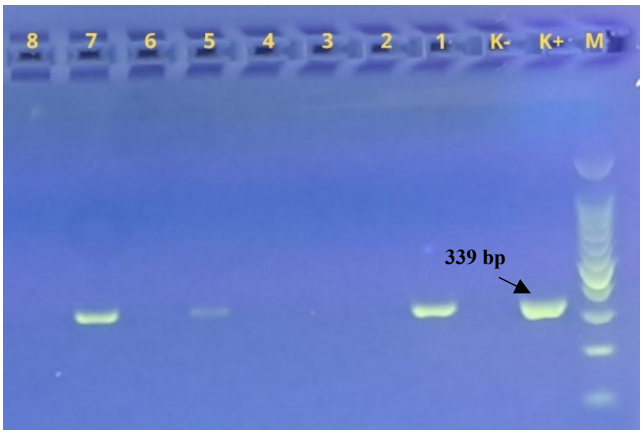

Figure 2. Agarose gel electrophoresis (2.0%) performed in 2023 showing PCR amplification of the *gyrA* gene (339 bp). Lane M: 100 bp DNA ladder (100-1500 bp), Lanes 1-8: Clinical samples, K^+: Positive control (purified *gyrA* amplicon from a *Porphyromonas gingivalis* clinical isolate previously confirmed by Sanger sequencing, GenBank accession: CP024591.1), K^-: Negative control (*Escherichia coli* ATCC 25922, non-target strain)

Table 6. Detection of the *gyrA* gene among study groups by PCR

Group	n	*gyrA* gene results (%)		p-value
		Positive	Negative	
Gold miners	30	19 (63.33)	11 (36.67)	0.02
Non-gold miners	30	10 (33.33)	20 (66.67)	
Total	60	29	31	

Table 7. Association between Hg concentration and bacterial detection, and association between bacterial variables

Analysis	Test	Statistic (U / χ^2)	Z	Effect size	p-value
Hg vs *Porphyromonas gingivalis*	Mann-Whitney	U = 322.5	-0.806	r = 0.10	0.420
Hg vs *gyrA*	Mann-Whitney	U = 446.0	-0.059	r = 0.01	0.953
Porphyromonas gingivalis vs *gyrA*	Chi-square	χ^2 = 22.191	-	φ = 0.61	<0.001

Age distribution also differed between the groups. Participants in the miner group were generally older (45-59 years), an age range associated with greater cumulative exposure to periodontal risk factors and age-related immunological changes. Aging has been shown to influence oral microbial ecology, inflammatory regulation, and host-microbiome interactions (Zhu et al. 2024). Although the overall mean age of the study population was 40.93±7.03 years, the imbalance in both age and sex between the groups may act as an important confounding factor. Because this study did not perform statistical adjustments for these demographic variables, the interpretation of microbiological and molecular comparisons should therefore be approached with caution.

The present study also demonstrated higher salivary mercury levels among gold miners compared with non-miners, reflecting occupational exposure to mercury commonly used in artisanal gold extraction. Despite this difference, all measured concentrations remained within a relatively low exposure range and did not exceed established salivary safety thresholds. Nevertheless, chronic low-dose exposure to mercury may still exert ecological effects on microbial communities. The substantial variability in mercury concentrations observed among miners suggests heterogeneous exposure patterns, which may depend on the intensity and duration of contact with mercury during mining activities (Palomares-Bolaños et al. 2025). Similar patterns of elevated mercury exposure have been reported in Artisanal and Small-scale Gold Mining (ASGM) communities worldwide, where chronic exposure occurs through inhalation of mercury vapor and contact with contaminated water or soil. In Indonesia, studies conducted in Gorontalo Utara have demonstrated that ASGM operations release substantial amounts of mercury into the environment, leading to contamination of water, sediments, and local food sources (Arifin et al. 2020). Likewise, investigations in Sukabumi reported elevated mercury concentrations in hair samples of residents near gold-processing facilities, indicating chronic environmental exposure associated with mining activities (Harianja et al. 2020). Collectively, these findings highlight the widespread environmental and public health risks associated with mercury use in small-scale gold mining.

Heavy metals such as mercury are known to influence microbial ecology through several mechanisms, including oxidative stress induction, disruption of redox balance, and selective pressure favoring metal-tolerant microorganisms (Könönen et al. 2022). These processes may alter microbial competition dynamics within periodontal biofilms and potentially influence community composition (Salam et al. 2019). In addition, environmental factors such as dietary habits, water sources, and fish consumption may contribute to mercury exposure even among individuals not directly involved in mining activities. However, the biological interpretation of the measured mercury concentrations remains limited because the present study did not assess mercury speciation, dose-response relationships, or biomarkers of toxicity (Wu et al. 2024).

Culture-based identification of anaerobic bacteria yielded a very low recovery rate in both study groups. Only

three isolates were identified in the miner group and one isolate in the non-miner group, including *P. denticola*, *P. melaninogenica*, and *Bifidobacterium* sp.. No *P. gingivalis* isolates were recovered using conventional anaerobic culture methods. The very low culture recovery observed in this study likely reflects both methodological limitations of anaerobic cultivation and the biological complexity of periodontal biofilms, in which many organisms are difficult to cultivate under standard laboratory conditions (Tanner 2015). Periodontal pathogens such as *P. gingivalis* are obligate anaerobes that are highly sensitive to oxygen exposure and environmental fluctuations during sample collection and laboratory processing (Phillips et al. 2018). Consequently, culture-based approaches alone are often insufficient to capture the full diversity of periodontal microbial communities (Progulske-Fox et al. 2022).

In contrast, PCR analysis detected *P. gingivalis* in the majority of participants across both groups. Molecular methods are known to provide higher analytical sensitivity compared with culture techniques and are capable of detecting bacterial DNA even when viable cells are present in low abundance or are difficult to cultivate (Ingalagi et al. 2022). Although the prevalence of *P. gingivalis* was higher among gold miners than non-miners, this difference was not statistically significant. The absence of statistical significance may partly reflect the moderate sample size of the study, which limits the ability to detect subtle group differences.

Another important finding was the significantly higher prevalence of the *gyrA* gene among gold miners. The *gyrA* gene encodes the A subunit of DNA gyrase, an essential enzyme involved in bacterial DNA replication. Importantly, the presence of the *gyrA* gene alone does not directly indicate fluoroquinolone resistance, as this gene is widely present in many bacterial species. Fluoroquinolone resistance typically arises from specific point mutations within the Quinolone Resistance-Determining Region (QRDR) of the *gyrA* gene rather than from the mere presence of the gene itself. Because the present study employed qualitative PCR to detect gene presence only, it was not possible to determine whether resistance-associated mutations were present (de Sousa Moreira Almeida et al. 2020). Therefore, the observed *gyrA* detection should be interpreted as the presence of a genetic marker within the microbial community rather than confirmed phenotypic resistance.

Environmental heavy metals have been widely reported to promote the co-selection of antibiotic resistance genes through overlapping stress-response pathways (Mazhar et al. 2021). Mercury exposure, for example, can trigger adaptive mechanisms such as efflux pump activation, enhanced oxidative stress tolerance, and increased horizontal gene transfer potential (Pathak et al. 2020; Huang et al. 2022; Tiwari et al. 2022). These mechanisms may facilitate the persistence or enrichment of resistance-associated genetic elements within microbial communities. Consequently, the higher prevalence of the *gyrA* gene observed among gold miners may reflect combined influences of environmental exposure and microbial ecological dynamics within periodontal biofilms.

Correlation analysis did not demonstrate a significant association between salivary mercury levels and the presence of *P. gingivalis* or the *gyrA* gene. However, the interpretation of these findings must consider the limited statistical power observed for these correlations (0.12-0.47). Low statistical power increases the risk of Type II error, meaning that a true association may remain undetected (Darling 2022). In contrast, the strong correlation observed between *P. gingivalis* and *gyrA* detection showed high statistical power (0.93-0.99), suggesting that the study was sufficiently sensitive to detect large effect sizes.

Several limitations should be acknowledged when interpreting the results of this study. The relatively small sample size may reduce the ability to detect modest associations between mercury exposure and microbial outcomes. In addition, the cross-sectional study design prevents causal inference regarding the relationship between mercury exposure and periodontal microbiota changes. The imbalance in demographic characteristics between the groups, particularly with respect to age and sex distribution, may also act as a confounding factor influencing microbial composition. Furthermore, bacterial detection was performed using qualitative PCR, which identifies the presence of target genes but does not quantify bacterial abundance or detect specific resistance-associated mutations.

Future studies should therefore incorporate quantitative molecular approaches to provide a more comprehensive understanding of the relationship between environmental exposure and periodontal microbiology. The use of quantitative PCR (qPCR) would allow accurate measurement of bacterial load, while metagenomic sequencing could enable detailed characterization of the periodontal microbiome and identification of resistance-associated mutations, including those within the quinolone resistance-determining region of the *gyrA* gene. In addition, future research should consider larger sample sizes, longitudinal study designs, and improved control of demographic and environmental confounders to clarify the complex interactions between heavy metal exposure, microbial ecology, and antimicrobial resistance in periodontal disease.

In conclusion, this study demonstrated that artisanal gold miners with chronic periodontitis exhibited higher salivary mercury levels than non-miners (median 0.09 µg/L vs. 0.03 µg/L), although all concentrations remained below the established salivary toxicity threshold. PCR detection of *P. gingivalis* was high in both groups and did not differ significantly. In contrast, the *gyrA* gene was detected more frequently among miners (63.33%) than non-miners (33.33%). Culture-based recovery of anaerobic bacteria was minimal, highlighting the limitations of conventional cultivation methods for detecting fastidious periodontal pathogens. These findings suggest that chronic low-level environmental mercury exposure may be associated with shifts in the subgingival microbial environment and the occurrence of resistance-associated genetic markers. From a public health perspective, environmental exposure to heavy metals should be considered a potential factor influencing antimicrobial resistance dynamics in periodontal disease.

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