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**COMPARISON OF CULTURABLE BACTERIAL COLONY COUNTS FROM
 CARIOUS CAVITIES BETWEEN ARTISANAL GOLD MINERS AND NON-
 MINERS: A CROSS-SECTIONAL STUDY**

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ABSTRACT,

Background: Artisanal and small-scale gold mining involves occupational and environmental exposures that may be associated with oral health conditions. However, information on culturable bacterial colony counts obtained from Carious cavities among artisanal gold miners compared with non-miners remains limited.

Purpose: This study aimed to compare culturable bacterial colony counts obtained from dental caries swab samples between artisanal gold miners and non-miners with dental caries. **Methods:** This comparative observational study used a cross-sectional approach. A total of 30 carious cavity swabs were collected from artisanal gold miners and non-miners. Samples were serially diluted up to 10^{-10} and inoculated on blood agar. Total viable bacterial count was determined using the Total Plate Count (TPC) method and expressed as CFU/mL. Presumptive identification was performed using Gram staining and catalase test. **Results:** Of 30 samples, 18 met the countable criteria (30–300 colonies), consisting of 11 samples from artisanal gold miners and 7 from non-miners. The miner group showed higher total viable count compared to non-miner group. Independent t-test showed $p = 0.605$ ($p > 0.05$), indicating no statistically significant difference in total viable bacterial count from carious cavities. **Conclusion:** There was no significant difference in total viable bacterial count from carious cavities between groups. Mercury exposure may affect oral microbiota composition rather than total quantity.

Keywords: Artisanal gold mining; Bacterial colony count; Dental caries; Oral microbiology.

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INTRODUCTION

Dental caries remains one of the most prevalent oral health problems worldwide and nationally. Based on the 2023 Indonesian Health Survey (Survei Kesehatan Indonesia/SKI), the national prevalence of dental caries reached 82.8%, while Central Kalimantan Province reported a prevalence of decayed or cavitated teeth of 41.8%.¹ Previous studies have demonstrated a considerable prevalence of dental caries and other oral health disorders, including periapical tissue diseases and high DMF-T index scores at the district level. These findings reinforce that dental caries continues to represent a significant public health concern in Central Kalimantan.²⁻⁵

Dental caries is a biofilm-mediated and diet-modulated disease involving ecological changes within the oral microbial community that lead to the demineralization of dental hard tissues. Acidogenic microorganisms metabolize carbohydrates and produce organic acids, resulting in a reduction of local pH and subsequent demineralization of enamel and dentin.^{6,7} These conditions promote microbial dysbiosis, characterized by an increased abundance of acidogenic and aciduric microorganisms within the biofilm. Although *Streptococcus mutans* is recognized as a major cariogenic bacterium, dental caries is now considered a polymicrobial

ecological disease influenced by various environmental factors that can modify bacterial colonization, microbial activity, and the progression of carious cavities.^{7,8,9}

Mercury (Hg) exposure represents an environmental factor that may influence oral microbial ecology, although its underlying mechanisms remain insufficiently investigated. Artisanal and small-scale gold mining (ASGM) is recognized as one of the major sources of mercury contamination, particularly through the use of mercury amalgamation in gold extraction processes. In Central Kalimantan, ASGM activities along the Kahayan River have contributed to environmental contamination through mercury release from mining operations. Suoth *et al.*¹⁰ reported the presence of mercury in various environmental media surrounding ASGM areas, while Martani *et al.*¹¹ detected mercury concentrations in water samples from the Kahayan River. These findings indicate potential environmental mercury exposure risks among communities living in ASGM-affected areas.

Mercury exposure among ASGM communities may occur primarily through occupational activities associated with gold processing, particularly among individuals involved in mercury amalgamation processes. Gold miners may experience substantial mercury exposure due to their occupational activities and direct contact with mercury-containing materials. A recent study among small-scale gold miners in Ramang Village, Pulang Pisau Regency, Central Kalimantan, reported detectable levels of mercury in saliva samples from miners and investigated their association with salivary pH and dental caries indicators. Although no statistically significant association between salivary mercury levels and DMF-T was observed, the detection of mercury in saliva provided evidence of biological mercury exposure among ASGM workers and highlighted the need for further investigation into other pathways through which mercury exposure may affect oral health.¹²

Beyond its direct toxic effects on host tissues, mercury exposure may also influence oral microbial ecology. The oral microbiome is a dynamic microbial community influenced by host-related and environmental factors. Alterations in microbial composition may contribute to oral disease development.¹³ Heavy metals can act as environmental stressors that modify microbial community structure through selective pressure, potentially affecting bacterial survival, abundance, and metabolic activity.¹⁴ Previous studies have demonstrated that environmental exposures may alter oral microbial composition and promote dysbiosis, which may influence bacterial characteristics associated with oral diseases, including dental caries.^{14,15} However, evidence regarding the relationship between mercury exposure and changes in culturable bacterial burden within carious cavity remains limited.

This knowledge gap is particularly relevant in ASGM communities in Central Kalimantan, where environmental mercury exposure and a high burden of dental caries coexist. Previous studies have documented mercury exposure among gold miners and described alterations in oral environmental factors; however, it remains unclear whether individuals involved in ASGM activities exhibit different bacterial colony counts within carious cavities compared with non-miners. Understanding these differences may provide preliminary insight into whether occupational mercury exposure is associated with changes in the culturable bacterial load of carious cavity.

Therefore, this study aimed to determine and compare culturable bacterial colony counts isolated from carious cavities between artisanal gold miners and non-miners in Central Kalimantan using a cross-sectional study design. The findings are expected to contribute preliminary evidence regarding the potential association between ASGM-related environmental exposure and oral microbial characteristics, supporting future investigations into environmental factors, oral microbiome alterations, and dental health risks among mining communities.

MATERIALS AND METHODS

This study employed a comparative observational design with a cross-sectional approach using dental caries swab specimens collected from artisanal and small-scale gold miners in Ramang Village, Pulang Pisau Regency, and non-miners in Palangka Raya City, Central Kalimantan. Potential confounding factors, including geographical location, socioeconomic status, dietary habits, oral hygiene practices, smoking behavior, water sources, and lifestyle factors, were considered in the interpretation of the findings. The miner group was recruited from an ASGM-affected area along the Kahayan River with potential mercury exposure, whereas the non-miner group was selected based on the absence of mining activities and river water use. Inclusion and exclusion criteria were applied to reduce potential confounding factors; however, matching between groups and statistical adjustments were not performed due to the limited sample size.

Sample size was determined using the Lemeshow formula because the target population size and preliminary data regarding bacterial colony counts were unavailable. With a 95% confidence level, $P = 0.5$, and a precision level of 25%, the minimum required sample size was estimated at 15.37 participants. The sample size was subsequently increased to 30 participants (15 miners and 15 non-miners) to achieve balanced allocation between the two groups. Since no previous data on effect size were available, an a priori power calculation was not performed. Therefore, this study was considered exploratory, and the findings should be interpreted while considering the limitation of the sample size.

$$n = \frac{Z^2 P(1-P)}{d^2}$$

A structured questionnaire was used to ensure participant eligibility. A total of 30 participants (15 artisanal and small-scale gold miners and 15 non-miners) aged 15–65 years were recruited. The miner group had **at least 5 years of mining experience** and used river water for daily activities, whereas the non-miner group had no history of mining occupation or river water use. Participants were excluded if they were receiving antibiotic therapy, using antiseptic mouthwash, wearing orthodontic appliances or dental prostheses, or unwilling to undergo sampling procedures. Bacterial swab samples were collected from moderate-to-deep carious cavities located on the posterior teeth of the maxillary or mandibular arches, which were clinically classified by a dentist. Lesion selection criteria and sampling procedures were standardized between both groups; however, radiographic evaluation and standardized caries scoring systems were not performed.

This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Palangka Raya, under ethical clearance number 64/UN24.9/LL/2024.

Bacterial Sample Collection from Dental Caries Cavity Using the Swab Method

Carious cavity samples were collected using a swab technique with the assistance of a dentist under aseptic conditions. Sterile cotton swabs moistened with 0.9% NaCl solution were applied to moderate-to-deep carious cavities located on posterior teeth of the maxillary or mandibular arches, with the sampling area isolated using cotton rolls to minimize saliva contamination. Lesion selection criteria and sampling procedures were standardized between both groups. The specimens were immediately transferred into Brain Heart Infusion (BHI) broth as a transport and enrichment medium for microbiological analysis. The interval between sample collection and laboratory processing was approximately 120 minutes.

Preparation of Blood Agar Medium

Blood agar medium was prepared by dissolving the *blood agar base* in distilled water until homogeneous, followed by heating and sterilization using an autoclave at **121°C for 15 minutes**. After cooling, sterile blood was aseptically added and mixed thoroughly, then the medium was poured into sterile Petri dishes and allowed to solidify before use.

Preparation of Brain Heart Infusion Broth (BHIB) Medium

Brain Heart Infusion Broth (BHIB) medium was prepared by weighing the required amount of BHIB powder and transferring it into an Erlenmeyer flask. Distilled water was added to the medium, and the mixture was stirred thoroughly until evenly dissolved. The medium was heated using a hotplate to achieve complete homogenization. After homogenization, the medium was aseptically dispensed into sterile test tubes and sealed with aluminum foil. The prepared medium was then sterilized in an autoclave at 121°C for 15 minutes. Following sterilization, the medium was cooled and incubated at 37°C for 24 hours to confirm media sterility prior to use.

Sample Dilution and Inoculation Procedures

All laboratory instruments were sterilized using an autoclave prior to the dilution procedure. Samples were prepared as bacterial suspensions and serially diluted using 0.9% NaCl solution up to a 10^{-10} dilution, with homogenization performed using a *vortex mixer*. The diluted samples were then inoculated onto blood agar medium, incubated at 37°C for 24–48 hours, examined for colony morphology and hemolytic characteristics, and quantified using a colony counter.

Gram Staining Procedure

Gram staining was performed using appropriate personal protective equipment. Bacterial colonies were fixed onto glass slides and stained with crystal violet, iodine/Lugol's solution, followed by decolorization with 95% alcohol and counterstaining with safranin. The stained preparations were examined under a light

microscope using immersion oil. Gram-positive bacteria appeared purple, whereas Gram-negative bacteria appeared red.

Catalase Test

The catalase test was performed using bacterial colonies grown on blood agar medium. Approximately 1–2 loopfuls of 3% hydrogen peroxide (H₂O₂) reagent were placed onto a glass slide. A single bacterial colony was then aseptically collected and mixed with the reagent. The test results were interpreted based on the presence or absence of air bubble formation on the slide, indicating catalase enzyme activity.

Data Processing and Statistical Analysis

Total viable bacterial counts were determined using the **Total Plate Count (TPC)** method on *blood agar* plates with an acceptable counting range of 30–300 colonies. Of the 30 collected samples, 18 samples were eligible for final analysis (11 artisanal gold miners and 7 non-miners). Statistical analysis was performed using individual sample values rather than cumulative colony counts. The data met the assumptions of normality (Shapiro–Wilk test) and variance homogeneity (Levene’s test); therefore, an **independent samples t-test** was performed, showing no statistically significant difference in total viable bacterial counts between the miner and non-miner groups ($t = -0.527$; $df = 16$; $p = 0.605$).

RESULTS

Total viable bacterial counts were determined using the Total Plate Count (TPC) method on *blood agar* plates inoculated from the 10⁻¹⁰ dilution, with plates meeting the 30–300 colony criteria included for quantitative analysis. Among the 30 collected samples, 18 samples met the analysis criteria (11 artisanal and small-scale gold miners and 7 non-miners), whereas the remaining 12 samples were excluded because the colony counts were outside the acceptable counting range. The limitations associated with using a single dilution level and unequal sample sizes between groups were considered in the interpretation of the findings. Statistical analysis was performed using individual values from valid samples rather than cumulative colony counts.

Table 1. Comparison of Bacterial Colony Counts Between Artisanal Gold Miners and Non-Miners

Group	Valid Samples (n)	Total viable bacterial count (×10 ¹⁰ CFU/mL), Mean ± SD	Mean difference (95% CI)	p-value	Interpretation
Artisanal gold miners	11	81.91 ± 40.08	-11.091 (-55.699 to 33.518)	0.605	Not statistically significant
Non-miners	7	93.00 ± 48.73			

Table 2. Morphological and Biochemical Characteristics of Bacterial Isolates from Artisanal Gold Miners with Dental Caries

Isolate	Colony Count at 10 ⁻¹⁰ dilution	Hemolysis	Gram Staining	Catalase Test
P 1	127	γ	Gram-positive clustered cocci	Positive
P 2	39	β, γ	Gram-positive cocci	Positive
P 3	32	γ	Gram-positive cocci	Positive
P 4	69	γ	Gram-positive chain bacilli	Positive
P 5	126	β, γ	Gram-negative chain bacilli	Positive
P 6	36	β, γ	Gram-negative chain bacilli	Positive
P 7	130	β, γ	Gram-positive cocci	Positive
P 8	48	β, γ	Gram-positive cocci and chain bacilli	Positive
P 9	103	β, γ	Gram-negative bacilli	Positive
P 10	71	β, γ	Gram-positive bacilli and clustered cocci	Positive
P 11	120	γ	Gram-negative cocci	Positive

Table 3. Morphological and Biochemical Characteristics of Bacterial Isolates from Non-Miners with Dental Caries

Isolate	Colony Count at 10 ⁻¹⁰ dilution	Hemolysis	Gram Staining	Catalase Test
NP 1	130	β, γ	Gram-positive cocci and chain bacilli	Positive
NP 2	88	γ	Gram-negative short-chain bacilli	Positive
NP 3	34	β, γ	Gram-positive bacilli and clustered cocci	Positive
NP 4	180	β, γ	Gram-negative cocci	Positive
NP 5	58	β, γ	Gram-positive chain bacilli	Positive
NP 6	92	β, γ	Gram-positive bacilli	Positive
NP 7	69	γ	Gram-positive clustered cocci	Positive

Bacterial isolates were characterized based on Gram staining morphology and catalase reactions, including Gram-positive and Gram-negative cocci and bacilli. These characteristics were considered presumptive and did not represent definitive species- or genus-level identification. Gram-positive cocci with positive catalase reactions suggested *Staphylococcus*-like phenotypes; however, other bacterial groups, such as *Streptococcus*, could not be excluded because further biochemical or molecular identification was not performed.

DISCUSSION

The absence of a statistically significant difference in bacterial colony counts between artisanal and small-scale gold miners and non-miners in the present study may have been influenced by several factors. Although the miner group demonstrated a higher overall bacterial colony count, variability in bacterial growth among samples and the relatively small number of valid samples may have affected the statistical power of the analysis. In this study, only 11 valid samples from the miner group and 7 valid samples from the non-miner group fulfilled the criteria for Total Plate Count (TPC) analysis, thereby limiting the ability of statistical tests to detect differences between groups.

In addition, dental caries is a multifactorial disease influenced by numerous factors, including oral hygiene, consumption of cariogenic foods, salivary pH, individual immune responses, and oral microbiota interactions. These factors may substantially contribute to bacterial growth and colonization in both study groups, thereby potentially masking any differences in bacterial colony counts between the two occupational groups. Previous studies have also reported no significant association between salivary mercury levels and dental caries experience measured using the DMF-T index among mining populations.¹²

Exposure to heavy metals, including mercury, is known to exert selective pressure on microbial communities through mechanisms involving toxicity, bacterial adaptation, and alterations in microbial community structure.^{16,17} Previous studies have suggested that exposure to heavy metals, including mercury, may influence microbial ecology through selective pressure mechanisms involving toxicity, bacterial adaptation, and changes in microbial community structure. Heavy metals may influence bacterial survival, select for microorganisms with tolerance mechanisms, and modify microbial interactions within microbial communities.^{16,17} However, these ecological effects cannot be directly extrapolated to the present study. Among artisanal gold mining populations, mercury exposure has been investigated in relation to oral health outcomes and specific oral microbial characteristics; however, the association between mercury exposure and alterations in oral microbial community composition remains unclear.¹⁸ The present study could not directly evaluate this relationship because individual mercury levels were not measured and microbial communities were not characterized using species-level or molecular approaches.

The findings of this study should be interpreted within the framework of the ecological plaque hypothesis, which proposes that dental caries development is associated not only with an overall increase in bacterial load but also with alterations in microbial community balance, interspecies interactions, and environmental changes within the oral biofilm.^{20,21,22} Consequently, the cultivable bacterial counts and basic phenotypic characteristics observed in this study represent only a subset of the oral microbial profile and do not fully reflect the overall composition and ecological changes of the oral microbiota.^{20,22,24}

Dental caries itself results from the demineralization of dental hard tissues caused by acids produced through the metabolism of dysbiotic oral biofilms. Recent evidence suggests that caries develops through

ecological shifts in multispecies oral biofilms, in which acidogenic and aciduric bacteria become dominant following frequent exposure to fermentable carbohydrates. Under such conditions, the genus *Streptococcus*, particularly *Streptococcus mutans*, plays a critical role as a cariogenic bacterium alongside other microorganisms such as *Lactobacillus*, *Actinomyces*, and several other *Streptococcus* species.^{6,7,8} Although these microorganisms are important contributors to cariogenesis, species-level identification of cariogenic bacteria was not performed in the present study. Furthermore, caries progression is influenced by microbial interactions, extracellular polysaccharide (EPS) production, and biofilm architecture that supports the establishment of acidic microenvironments on tooth surfaces.

The Gram staining and catalase test results showed that several bacterial colonies exhibited Gram-positive coccoid morphology with positive catalase reactions, suggesting presumptive *Staphylococcus*-like phenotypes. However, these findings were based only on phenotypic characteristics and do not represent definitive genus-level identification. *Staphylococcus* species have been reported as members of the oral microbial community and may act as opportunistic microorganisms under certain conditions. Previous studies by Satrio et al.²⁴ have reported the isolation of *Staphylococcus* species from dental caries cavity; however, species-level identification was not performed in the present study.

Although *Streptococcus mutans* is widely recognized as the primary cariogenic pathogen, dental caries development is actually influenced by the complexity of microbial interactions within the oral biofilm. Several commensal species, such as *Streptococcus gordonii* and *Streptococcus oralis*, have been reported to inhibit the growth of *S. mutans* through hydrogen peroxide (H₂O₂) production, thereby reducing the cariogenic potential of the biofilm.²⁵ However, alterations in the oral environment, including high sugar consumption and potential environmental exposures, may shift the microbial balance toward a more cariogenic community.

In addition to microbiological factors, dental caries is also influenced by various other health determinants, including socioeconomic status, malocclusion, oral hygiene practices, fluoride exposure, and the quality of water used in daily life. These factors are presumed to contribute to the high prevalence of dental caries in the Indonesian population and may also have influenced the findings of the present study.²⁶⁻³⁰

The findings demonstrate that there was no statistically significant difference in culturable bacterial colony counts between artisanal and small-scale gold miners and non-miners. However, this finding should not be interpreted as evidence that mercury exposure has no influence on oral microbial characteristics, because individual mercury exposure was not directly measured and microbial community composition was not comprehensively assessed. The observed differences should therefore be interpreted as variations in culturable bacterial counts and basic phenotypic characteristics between occupational groups.

The presence of Gram-positive coccoid colonies with catalase-positive reactions observed in this study reflects the diversity of culturable bacterial phenotypes associated with dental caries under the applied culture conditions. These findings highlight the complexity of the oral microbial ecosystem, in which dental caries is influenced not only by bacterial quantity but also by microbial interactions, biofilm ecology, environmental conditions, and host-related factors. Consistent with the ecological plaque hypothesis, dental caries is not solely associated with increased bacterial load but involves ecological changes within the oral biofilm. Therefore, oral health outcomes among mining populations are likely shaped by multifactorial determinants, including oral hygiene practices, dietary habits, socioeconomic factors, and environmental exposures.

Further studies with larger sample sizes, direct assessment of mercury exposure, and advanced molecular microbiological approaches are required to better elucidate the potential relationship between mercury exposure, oral microbial composition, and dental caries progression in artisanal and small-scale gold mining communities.

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