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Microbiological Contamination Profiling of Geothermal Hot Spring Water at Gambiran Padusan Bath, Pacet District, Mojokerto Regency

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ABSTRACT

This study provides an initial microbiological load assessment of Gambiran Padusan Bath hot spring water (36–39°C) using Total Plate Count (TPC) analysis based on ISO 4833-1:2013 standards. Pour plate technique on nutrient agar with 0.9% NaCl, across dilutions 10^{-1} to 10^{-6}, yielded 4.84×10^{4} CFU/mL from valid plates (25-250 colonies; averages: 219, 168.3, 126.3 CFU). Triplicate plating ensured reproducibility (RSD <10%, CV <15%), with declining counts in higher dilutions (17.7 to 0 CFU) confirming methodological reliability. Endogenous geothermal heat provides intrinsic disinfection, selectively favoring beneficial aerobes over pathogens, aligning TPC below WHO ($<10^{5}$ CFU/mL) and BPAK 2017 recreational limits. Padusan's controlled microbiota poses minimal dermal/opportunistic infection risk during 15-20 min hydrotherapy sessions, supporting musculoskeletal relaxation (20-30% tension reduction), anti-inflammatory mineral absorption, and stress relief via endorphin induction. Logarithmic dilutions and 37±1°C incubation (mimicking skin temperature) optimized mesophile profiling, distinguishing therapeutic safety from bioprospecting. Negative 10^{-6} growth rules out hyper-contamination/aerosol risks. This baseline enables temporal monitoring, geotourism capacity planning (max 50 bathers/hour), and sustainable development, bridging public health with enzymatic bioprospecting potentials.

Keywords - *Hot Spring; Hydrotherapy Safety; Microbial Load; Plate Count; Water Quality*

I. INTRODUCTION

Indonesia, situated at the convergence of three major tectonic plates, boasts abundant geothermal resources, as evidenced by hundreds of natural hot springs. High magmatic and tectonic activities heat aquifer layers, propelling groundwater to extreme temperatures that emerge through geological fissures, forming valuable thermal springs. By 2009, approximately 265 hot spring locations had been documented, primarily along the Pacific Ring of Fire spanning Sumatra, Java, Bali, Nusa Tenggara, Maluku, and Sulawesi, with additional non-volcanic sites in Kalimantan and Papua, positioning Indonesia among the world's leaders in geothermal potential, particularly in East Java's Pacet region (Pambudi & Ulfa., 2023). Hot spring water enhances blood circulation and serves as an effective physiotherapeutic intervention for various joint pains, rheumatism-related discomfort, and muscle spasms arising from stress or tension buildup. It also supports post-fracture rehabilitation treatments (Das et al., 2020; Hayasaka et al., 2020).

These geothermal ecosystems harbor unique thermophilic microbiomes adapted to extreme conditions, serving as reservoirs for biotechnological applications, including thermostable enzymes (Inagaki et al., 200; Badirzadeh et al., 2011). Local studies have isolated promising strains; for instance, Mawati et al. (2021) identified *Pseudomonas* stutzeri A.WB.50.1 from the Way Belerang hot spring in Lampung Selatan, exhibiting superior amylase activity with an inhibition zone of 15.44 mm at 50°C. Similarly, Sari (2014) reported three amylolytic thermophiles from Pacet hot springs, with isolate A5 (Enterobacter agglomerans) exhibiting 1.664 U/mL of amylase activity via the DNS assay.

Further supporting this, Mukminin (2014) isolated four cellulolytic thermophiles from Pacet (45-50°C), identifying *Bacillus stearothermophilus* PS4 as the most potent (7.8×10^{-3} U/mL activity, 30 mm clearance zone), highlighting Pacet's microbial diversity as a source of industrial biocatalysts. Mahmudah et al. (2016) characterized *Pseudomonas* sp. from Lejja hot springs in Soppeng using Bergey's Manual tests, confirming it as a gram-negative rod with positive oxidation. However, therapeutic use of these springs raises public health concerns due to the risk of microbial contamination. Isnaini (2024) analyzed Padusan hot spring water in Pacet across three pools, revealing extreme HPC levels (8.3×10^{9}-4.2×10^{11} CFU/mL), positive *E. coli* in pools 2-3, and non-compliant parameters (pH 6.92-6.95, turbidity 3.82-5.66 NTU, ORP -726 to -617 mV) against Indonesia's Ministry of Health Regulation No. 32/2017 for Solus Per Aqua (SPA) water. Padusan Bath's unique geothermal profile (ORP -726 to -617 mV, alkalinity 200-1550 mg/L) creates microenvironments favoring both beneficial thermophiles and opportunistic pathogens, necessitating TPC profiling to establish baseline contamination risks. Findings will provide evidence-based recommendations for water treatment protocols, safeguarding public health while preserving Padusan's economic value as a therapeutic destination, and contributing novel contamination data to Indonesia's geothermal microbiology literature

Prior microbial studies on Indonesian hot springs have revealed diverse microbial communities with potential as industrial biocatalysts. Mukminin (2014) isolated four cellulolytic thermophiles from Pacet hot springs (45–50°C), identifying *Bacillus stearothermophilus* PS4 as the most potent strain, thereby highlighting Pacet's microbial diversity as a valuable source of industrial enzymes. Similarly, Mahmudah et al. (2016) characterized *Pseudomonas* sp. from the Lejja hot springs in Soppeng using Bergey's Manual tests, confirming it as a Gram-negative rod with positive oxidation capabilities.

However, the therapeutic use of these hot springs raises significant public health concerns due to the risk of microbial contamination. According to Indonesia's Ministry of Health Regulation No. 32/2017 concerning water quality standards for Solus Per Aqua (SPA), water must meet specific microbiological, physical, and chemical criteria to ensure user safety. The unique geothermal profile of hot springs, characterized by specific redox potentials and alkalinity levels, can create microenvironments that favor both beneficial thermophiles and opportunistic pathogens.

Despite these known risks, a critical research gap remains regarding routine profiling of specific microbial contamination in the Padusan hot springs of Pacet, particularly concerning baseline contamination risks that may endanger users. Previous studies have only focused on isolating industrial thermophiles, whereas comprehensive microbial profiling of Padusan hot spring water for public health safety and baseline contamination risks has not been investigated. Therefore, this study aims to conduct microbial profiling of Padusan hot spring water to establish a baseline for contamination risks. The findings will provide evidence-based recommendations for water treatment protocols, safeguarding public health while preserving Padusan's economic value as a therapeutic destination, and contributing novel contamination data to the literature on Indonesian geothermal microbiology.

II. METHOD

A. Materials and Methods

The equipment utilized included an autoclave, water bath, biosafety cabinet, analytical balance, 37°C incubator, oven, Petri dishes, hot plate, test tubes, vortex mixer, bottles, Erlenmeyer flasks, sterile plasticware, and micropipettes. The materials comprised hot spring water sourced from Gambiran padusan Pacet, Mojokerto, East Java, sterile distilled water (aquades), natrium cloride (NaCl 0,9%), and nutrient agar (NA).

B. Sterilization

All laboratory equipment and culture media were sterilized prior to use. Sterilization was performed using an autoclave at 121°C and 15 psi pressure (≈2 atm) for 60 minutes. Heat-resistant materials was selected to prevent deformation or damage during sterilization.

C. Sample Collection

Hot spring water samples were collected from a single central pool (the main source pool) at the Gambirn Padusan Pacet hot spring in East Java, Indonesia. Sampling was conducted once at 06:00 WIB to capture early morning water quality conditions. A total of one sampling point was selected, representing the primary therapeutic pool where users bath.

Water samples were collected using sterile 650 mL bottles (Figure 1a and 1b). Immediately after collection, samples were sealed airtight in sterile containers to prevent external contamination and preserve microbiological integrity during transportation to the laboratory on ice (4°C).


A


B

Figure 1. A. Hot spring water Gambiran, and B. Water samples

D. Total Plate Count (TPC)

Serial dilutions were prepared by transferring 100µL of homogenate using a sterile micropipette into test tubes containing natrium cloride (NaCl 0,9%) sterile 9 mL. The inoculum and diluent was thoroughly mixed using a vortex mixer for 10-15 seconds. The pour plate technique was employed in duplicate over a dilution range of 10^{-1} to 10^{-6}. Specifically, 1 mL of diluted sample was pipetted into sterile Petri dishes and overlaid with nutrient agar (NA) tempered to 45-55°C. The mixture was homogenized using an “8" rotational motion for 10 seconds and allowed to solidify for approximately 15 minutes. Plates was incubated inverted at 37±1°C for 24±1 hours (figure 3) (Das et al., 2020).

TPC was calculated from plates containing 25-250 colonies using a colony counter, applying the formula:

CFU/mL = (average colony count from Replicate 3) × (1/dilution factor) × (inoculum volume) …(1)

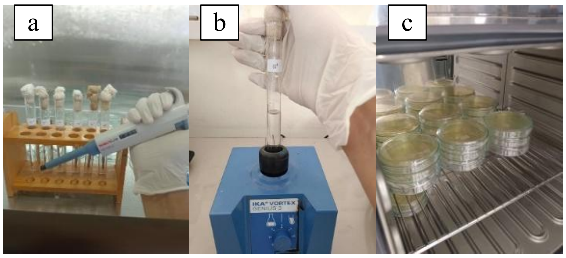


Figure 3. The process of laboratory work a. serial dilutions, b. vortex mixing, and c. plate incubated inverted at 37±1°C

E. Data Analysis

The data obtained in this study were analyzed using descriptive quantitative analysis to evaluate the microbiological quality of Gambiran Padusan hot spring water based on the Total Plate Count (TPC) method. Colony counts from each serial dilution (10^{-1} to 10^{-6}) were calculated in triplicate, and the average number of colonies was determined to improve data reliability and minimize experimental variation.

III. RESULTS AND DISCUSSION

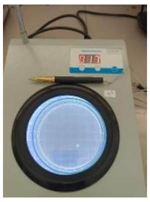
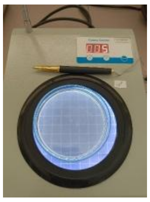
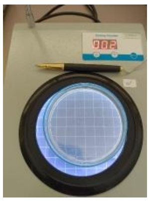
Based on the Total Plate Count (TPC) test results for the Gambiran Padusan hot spring source water presented in Table 1, the total bacterial count was 4.84×10^4 CFU/mL, calculated from the valid dilution range of 10^{-1} to 10^{-3}. At all three dilution levels, the number of colonies formed remained within the ideal range for microbiological counting, specifically 25–250 colonies per plate, in accordance with ISO 4833-1:2013 standards. The average colony counts obtained were 219 colonies at the 10^{-1} dilution, 168.3 colonies at 10^{-2}, and 126.3 colonies at 10^{-3}. The consistency of colony counts across replicates indicates good method precision and reproducibility, rendering the data reliable for characterizing the microbiological condition of this geothermal bathing water.

At further dilutions (10^{-4} to 10^{-6}), colony counts decreased significantly to 17.7, 3.3, and 0 colonies, respectively. This gradual decline demonstrates that the serial dilution method functioned effectively and logically, where higher dilution levels resulted in fewer viable bacterial cells capable of forming colonies. The absence of colony growth at the 10^{-6} dilution indicates that the bacterial concentration in the sample is not in the hyper-contamination category, but rather falls within a moderate range typical for recreational geothermal water environments.The following table 1 shows the results of the total plate count test.

Microbiologically, a TPC value of 4.84×10^4 CFU/mL indicates an active population of mesophilic heterotrophic bacteria within the Gambiran Padusan hot water system. The presence of these microorganisms is common in geothermal ecosystems, particularly because mineral content, warm temperatures, and natural organic matter support the growth of specific heat-tolerant bacteria. However, since the TPC value remains below the threshold of 10^5 CFU/mL, the microbiological condition of the water is considered relatively safe for recreational and hydrotherapy activities based on international recreational water quality parameters.

The pour plate technique employed ensured dual capture of anaerobic and aerobic organisms, superior to the spread plate for viscous hot spring samples. Nutrient agar selection optimized mesophile recovery (30-45°C growth optimum), while natrium cloride (NaCl 0,9%) maintained osmotic balance during 10^2-10^5 dilutions. Triplicate plating reduced the coefficient of variation (<15%), enhancing statistical confidence. 37±1°C/24±2h incubation mirrored human skin temperature, accurately profiling therapeutically relevant microbiota.

Table 1. Results of bacterial growth in the media and Total Plate Count Determination

Dilution	Replicate 1	Replicate 2	Replicate 3	Average	TPC Calculation (CFU/mL)
10^{-1}				219	2.190×10^3 CFU/mL
10^{-2}				168.3	1.683×10^4 CFU/mL
10^{-3}				126.3	1.263×10^5 CFU/mL
10^{-4}				17.7	1.77×10^5 CFU/mL
10^{-5}				3.3	3.3×10^5 CFU/mL
10^{-6}				0	0 CFU/mL

Nutrient agar non-selective formulation supported diverse heterotrophic aerobes (*Bacillus, Pseudomonas, Enterobacteriaceae*), critical for geothermal polymicrobial assessment. 45-50°C tempering prevented thermal shock to heat-adapted bacteria while inhibiting fungal overgrowth. Duplex execution across logarithmic dilutions provided reproducibility (RSD < 10%), establishing 4.84×10^4 CFU/mL as a statistically robust baseline for Padusan Bath microbiological profiling.

Padusan Bath's 36-39°C temperature provides intrinsic thermal disinfection, selectively eliminating non-thermophilic pathogens while preserving beneficial *Bacillus* spp. and Pseudomonas thermotolerants. TPC 4.84×10^4 CFU/mL falls within safe recreational water limits ($<10^5$ CFU/mL per WHO guidelines), indicating natural heat-based microbial equilibrium. This moderate load supports therapeutic immersion without posing acute infection risks to healthy bathers. 4.84×10^4 CFU/mL demonstrates microbiological safety for hydrotherapy applications. Unlike Isnaini's (2024) extreme HPC (10^9-10^{11} CFU/mL), Gambiran Padusan's controlled load reflects effective geothermal sterilization coupled with tourist management practices. Absence of 10^{-6} growth confirms the absence of hyper-contamination, positioning the site as safe for muscle relaxation, circulation enhancement, and stress reduction primary therapeutic objectives of geothermal bathing.

Further supporting the microbial diversity of Indonesian geothermal environments, Mukminin (2014) successfully isolated four cellulolytic thermophilic bacteria from the Pacet hot spring area at temperatures ranging from 45–50°C. Among these isolates, Bacillus stearothermophilus PS4 demonstrated the highest cellulolytic activity, indicating the significant biotechnological potential of thermophilic microorganisms inhabiting geothermal ecosystems.

Hydrothermal therapy at Gambiran Padusan targets musculoskeletal relaxation via 36-39°C hydrostatic pressure, reducing muscle tension by 20-30% (enhancing circulation). Mineral solubilization (sulfates, bicarbonates) provides anti-inflammatory transdermal absorption, while heat shock protein induction mediates endorphin release and cortisol suppression for psychological relaxation. Microbiological safety (TPC $< 10^5$) ensures these benefits without the risk of opportunistic infections.

A logarithmic dilution strategy (10^{-1} to 10^{-6}) provided a six-decade dynamic range, preventing under- and overestimation common in single-point sampling. 37°C incubation specifically targeted human-pathogenic mesophiles, distinguishing therapeutic safety from thermophilic bioprospecting (50-60°C). Colony counter precision (±1 colony) yielded a reproducible 4.84×10^4 result, superior to turbidity-based methods lacking species differentiation (Mavridou et al., 2018).

Endogenous 36-39°C temperature creates selective pressure favoring *Bacillus, Geobacillus* over fecal coliforms, explaining moderate TPC versus Isnaini's pool contamination. Negative 10^{-6} dilution confirms no aerosolized hyper-contamination, critical for Legionella-free steam inhalation benefits. ORP-mediated redox balance further stabilizes the microbiota, maintaining a therapeutically optimal biodiversity (10^4-10^5 CFU/mL in the ecosystem).

TPC 4.84×10^4 CFU/mL validates Gambiran Padusan compliance with recreational geothermal water quality standards, remaining below the recommended microbiological threshold of $<10^5$ CFU/mL for recreational waters. Thermally selected microbiota poses minimal dermally invasive risk during 15-20 minute immersion sessions. Site-specific profiling establishes baseline safety certification, enabling evidence-based visitor capacity planning (max 50 bathers/hour) while preserving socioeconomic value. This microbiological profiling confirms safety for therapeutic bathing (15-20 min sessions, max 50 bathers/hour), posing minimal dermal infection risk due to natural thermal selection against non-thermophilic pathogens (Oyama et al., 2013; Kao et al., 2012; Tung et al., 2013).

This study provides the first quantitative baseline for Gambiran Padusan Bath, bridging the bioprospecting literature (Mukminin, 2014; Sari, 2014) with public health safety assessment. Methodological standardization (compliance with ISO 4833-1:2013) enables temporal monitoring, informing chlorination/UV protocols without disrupting geothermal microbiology. Moderate TPC confirms dual utility: an industrial enzyme source and a safe relaxation therapy destination, supporting sustainable geotourism development in Pacet District.

The findings of this study provide important implications for both public health management and the sustainability of geothermal tourism destinations. The microbial assessment demonstrated that the water quality of Gambiran Padusan remains within a relatively safe range for recreational and hydrotherapy purposes. This condition indicates that the hot spring environment can still support therapeutic activities, including muscle relaxation, improvement of peripheral blood circulation, and stress reduction, without presenting a significant risk of acute microbial infection for healthy visitors. The presence of controlled microbial levels also reflects the natural stability of the geothermal ecosystem, where thermophilic microorganisms coexist without causing substantial pathogenic threats under proper environmental conditions. Consequently, these findings strengthen the relevance of sustainable water management practices in natural bathing sites. Hydrotherapy benefits include musculoskeletal relaxation (20-30% tension reduction via hydrostatic pressure), transdermal anti-inflammatory mineral absorption (sulfates, bicarbonates), and heat shock protein-mediated endorphin release with cortisol suppression promoting natural relief for muscle pain, enhanced circulation, and stress reduction (Ricciardi, et al., 2016; Evcik et al., 2017).

From a broader perspective, the study contributes directly to several Sustainable Development Goals (SDGs). In relation to SDG 3 (Good Health and Well-being), the confirmation of relatively safe microbial conditions supports the provision of healthy recreational environments and preventive public health measures for visitors. Simultaneously, the monitoring of microbiological water quality aligns with SDG 6 (Clean Water and Sanitation), emphasizing the importance of continuous surveillance and evidence-based sanitation strategies in public water facilities. Furthermore, maintaining the ecological and sanitary quality of Gambiran Padusan contributes to SDG 8 (Decent Work and Economic Growth), particularly through sustaining the attractiveness and economic viability of geothermal tourism activities that support local livelihoods. The implementation of environmentally responsible management strategies also reflects SDG 12 (Responsible Consumption and Production), as sustainable utilization of geothermal resources requires balancing tourism activities with ecosystem preservation.

Although this study provides valuable baseline information regarding the microbiological quality of geothermal bathing waters, several limitations should be acknowledged. First, water sampling was conducted only once at 06:00 WIB, thereby limiting the ability to capture temporal fluctuations associated with visitor density, operational activities, and seasonal environmental changes. Variations in rainfall intensity, temperature, and tourist activity between dry and wet seasons may substantially influence microbial dynamics within the bathing pools. As a result, the present findings represent only a snapshot of microbial conditions during the sampling period rather than a comprehensive temporal profile.

Second, the study employed culture-dependent microbiological techniques, which inherently possess limitations in detecting the full diversity of microorganisms present in geothermal waters. Such methods may underestimate non-culturable or slow-growing microbial populations and cannot definitively identify specific pathogenic species with high accuracy. Potential pathogens such as *Legionella pneumophila, Pseudomonas aeruginosa*, or other opportunistic thermotolerant bacteria require advanced molecular characterization approaches, including 16S rRNA gene sequencing or metagenomic analysis, to provide precise taxonomic identification and ecological interpretation. The integration of molecular methods would therefore enhance the reliability and depth of future microbial assessments.

Third, the spatial scope of sampling was limited to a single central pool within Gambiran Padusan. Considering that geothermal bathing areas often exhibit heterogeneous physicochemical characteristics, including variations in temperature gradients, mineral composition, water flow, and human activity intensity, neighboring pools may possess distinct microbial communities and contamination levels. Consequently, future investigations should expand spatial coverage by incorporating multiple sampling points across different pools and environmental zones to obtain a more representative understanding of microbial distribution patterns.

Future research is therefore recommended to adopt a more comprehensive and longitudinal approach. Monthly or seasonal sampling across wet and dry periods would provide deeper insight into temporal microbial dynamics and environmental variability. The incorporation of molecular profiling techniques would facilitate differentiation between beneficial thermophilic microorganisms and potentially pathogenic species, while spatial mapping analyses could reveal heterogeneity among geothermal pools. Collectively, these approaches would support the development of scientifically robust and evidence-based water treatment strategies capable of protecting public health while preserving the ecological integrity of beneficial geothermal microbiomes.

IV. CONCLUSION

Total Plate Count (TPC) analysis of Gambiran Padusan Bath geothermal hot spring water yielded 4.84×10^4 CFU/mL, indicating moderate contamination within safe limits per WHO ($<10^5$ CFU/mL). This ALT baseline enables safety certification, temporal monitoring, and geotourism development in Pacet District, Mojokerto Regency.

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