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Airborne Microbial Assessment and Its Implication for Laboratory Safety

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ABSTRACT

The impact of airborne microbes on laboratory workers is substantial, as exposure to elevated bioaerosol concentrations can lead to respiratory illnesses, allergic sensitization, and an increased risk of laboratory-acquired infections. The air quality in microbiological laboratories is therefore a critical component of occupational health and safety. Previous studies have shown that microbial levels in educational laboratories frequently exceed international and national guidelines. Despite increasing recognition of the importance of indoor air quality, limited data are available on microbiological laboratory conditions outside Java, particularly in South Kalimantan. This study employed a descriptive, cross-sectional, observational design using the midget impinger method to collect air samples at two sampling points in each laboratory before and after ventilation activation, resulting in a total of 12 samples. Airborne bacterial counts were used to quantify microbial load, while temperature and relative humidity were simultaneously measured. Data were analyzed descriptively, and pre–post ventilation differences were assessed using the Wilcoxon Signed-Rank test. All microbial loads remained below the WHO (500CFU/m^3) and Ministry of Health Republic Indonesia (700CFU/m^3) thresholds. Three laboratories which relied solely on natural ventilation, exhibited the highest microbial counts, whereas laboratories with mechanical ventilation showed consistently lower levels. Although no significant differences were observed between pre-post ventilation conditions, naturally ventilated spaces tended to show higher microbial loads. Overall, airborne microbial levels and environmental parameters across the three laboratories remained within acceptable limits. However, higher humidity was associated with higher microbial concentrations, underscoring the importance of maintaining indoor environmental conditions within recommended ranges to ensure laboratory safety.

INTRODUCTION

The quality and concentration of airborne microbial loads in microbiology laboratories are crucial factors affecting occupational health and biosafety regulations. Indoor airborne microorganisms, commonly called bioaerosols, consist of bacteria, fungus, and viruses suspended in the air, originating from human activities, laboratory processes, and environmental factors. Exceeding the established standard for indoor air microbes can lead to respiratory illnesses, allergy responses, and potential laboratory-acquired infections (Umana et al., 2018). This concern is particularly significant in teaching laboratories, where frequent human presence and routine handling of cultures increase the risk of cross-contamination between individuals and experimental materials.

Recently, experiments have shown that microbial levels in educational and research laboratories frequently exceed acceptable safety limits. Investigations in academic settings have

reported airborne bacterial concentrations ranging from 10^2 to 10^3CFU/m^3, predominantly comprising Staphylococcus and Bacillus species, as well as fungal genera such as Aspergillus and Penicillium, which are also commonly detected (Giri, 2020; Mazlan et al., 2020). These findings often surpass both the WHO's recommended 500 CFU/m^3 limit and Indonesia's Ministry of Health standard of 700 CFU/m^3 (Kementerian Kesehatan Republik Indonesia, 2023). Although there is much attention to indoor air quality, data on teaching laboratories are rare, especially outside Java. In Campus X, South Kalimantan, the microbiology laboratory facilities in this study feature distinct ventilation designs and floor layouts, leading to variations in indoor airborne microorganisms. The goals of the present project are to explore levels of airborne microbial load and the environmental factors that influence them across three microbiology laboratories.

METHOD

This investigation employed a descriptive, observational, cross-sectional design to evaluate microbiological air quality and determine environmental factors affecting microbial counts in educational laboratories. The study was conducted in three microbiology laboratories at South Kalimantan University, Indonesia, with data collection in July 2025.

The media that absorbs air utilizing a 0.85 % NaCl Solution (Merck, 1.06404.1000). This solution was prepared by dissolving 0.85 grams of NaCl in 100 mL of distilled water. Then transfer 10 mL of the solution into a test tube and cover it with a cotton plug. Plate Count Agar (PCA) by Merck (product number 1.05463.0500) was used as a bacterial growth medium. This medium was made by weighing 22gr into 1 liter of distilled water and boiling until the substance became transparent. These two media are sterilized at 121^0C for 15 minutes.

Purposive sampling was applied to this research. Air samples were collected at the midpoint of each laboratory to ensure representative microbial counts. Air samples were collected using a midget impinger method (active method) at a constant airflow rate of 2 lpm. The sampled air was directed into a midget impinger containing 10 mL of 0.85% NaCl solution, which served as the collection medium. Sampling was performed for 30 minutes. Air sampling was performed at two points in each laboratory, before and after activation of the ventilation system.

Following sampling, 1mL of the NaCl solution containing the entrapped bacteria was transferred into a sterile Petri plate. This step is performed sterilely and aseptically in a Laminar Air Flow (LAP) or Biosafety Cabinet (BSC). PCA was subsequently poured into the plate, mixed gently, and permitted to solidify. Incubate bacteria in aerobic conditions using an inverted plate at 37°C for 48 hours (2×24 hours). The growth of microbes was enumerated by counting the number of colonies with a circular shape, milky-white color that appeared within the agar medium (subsurface colonies), rather than on its surface. The airborne microbial load is stated in CFU/m^3. It is by this equation (Kementerian Kesehatan Republik Indonesia, 2002):

$$R\ (\text{koloni/ml}) = \frac{(a-e) + (b-e) + (c-e) + (d-e)}{4}$$

$$JK = \frac{R \times V \times 1000/M^3}{Q \times t}$$

Where,

JK	=	The airborne microbial loads	t	=	Time of sampling (minutes)
R	=	The average of the colony bacteria	a-d	=	The colony bacteria on petridish a,b,c,d
V	=	Volume of NaCl (mL)	e	=	The colony of bacteria on the petridish control
Q	=	Flow rate (L/minute)			

Environment variables, including temperature (T) and relative humidity (RH), were collected at identical air sampling sites to assess factors that may influence microbial air quality. It was recorded using a calibrated thermohygrometer. Measurements were conducted before and after activation of the ventilation system. All measured parameters—including T, RH, and JK—

were initially summarized using descriptive statistics. The normality of the data was assessed using the Shapiro-Wilk test to determine the appropriate statistical approach. To evaluate differences between pre- and post-activation of the ventilation system by the Wilcoxon Signed Rank and Spearman rank for the correlation test. This study received ethical clearance from the University's Health Research Ethics Committee (KEPK) of the Poltekkes Kemenkes Banjarmasin under letter No. 060/KEPK-PKB/2025. In addition, formal authorization for sample collection was also obtained. Sample selection was carefully carried out in accordance with applicable regulations. Measurements were conducted in three laboratories.

RESULTS

A total of three microbiology laboratories were evaluated for airborne microbial loads and environmental parameters (T, RH) under pre- and post-ventilation conditions. Results are presented to compare environmental conditions and microbial loads across laboratories, and to examine how these findings relate to compliance with Indonesian Ministry of Health standards (Kementerian Kesehatan Republik Indonesia, 2016; 2023) and World Health Organization (Chawla et al., 2023; Kim et al., 2018). The comprehensive measurements of environmental parameters and microbial acquisition throughout the study are encapsulated in Table 1.

Table 1. Comparison of environmental conditions and the airborne microbial loads observed pre and post-ventilation system in microbiology laboratories

No	Lab Code	Space area (m^2)	Ventilation system (unit)	Position	Condition	The result of the measurement		
						T (^{0}C)	RH (%)	JK (CFU/m^3)
1	Lab 1	60	AC split (3)	Ground floor	Pre	30.4±0.39^a	62.8±5.25^a	9.4 x 10±62.51^a
2					Post	26.6±0.20^a	46±1.15^a	9.4 x 10±71.16^a
3	Lab 2	100	AC central (2)	3rd floor	Pre	30.4±0.24^a	60±5.23^a	8.3 x 10±0.02^a
4					Post	28.2±1.09^a	44±2.94^a	6.3 x 10±72.17^a
5	Lab 3	110	Window	5th floor	Pre	30.5 ±0.57^a	57±5.61^a	1.4 x 10^2±109.57^a
6					Post	29.6±0.57^a	58±2.94^a	1.1 x 10^2±62.49^a

Remarks: Lab 1,2,3=Laboratory 1,2,3, AC=Air Conditioning, Pre=Condition before activation of ventilation system, Post= Condition after activation of ventilation system, T=Temperature, RH=humidity, JK=The airborne microbial loads.

Table 2. The result of descriptive statistic

Parameter	Lab	Normality p-value		Wilcoxon p-value
		Pre	Post	
Temperature	Lab 1	0.408	0.001	0.068
Temperature	Lab 2	0.488	0.316	0.066
Temperature	Lab 3	0.272	0.512	0.144
Humidity	Lab 1	0.041	0.024	0.068
Humidity	Lab 2	0.145	0.734	0.068
Humidity	Lab 3	0.858	0.348	1.000
Microbial Load	Lab 1	0.224	0.850	0.715
Microbial Load	Lab 2	0.024	0.195	0.715
Microbial Load	Lab 3	0.369	0.224	0.655

The Shapiro–Wilk test results in Table 2 show that some pre- and post-measurement values did not follow a normal distribution, particularly in the relative humidity and microbial load datasets, as indicated by p-values below 0.05. Because several variables violated the assumption

of normality, non-parametric statistical methods were applied for subsequent analyses. The Wilcoxon Signed-Rank test also showed no statistically significant differences between pre- and post-ventilation conditions across all laboratories for temperature, relative humidity, and microbial load ($p>0.05$).

Table 3. The combined result of normality and the Wilcoxon test

Parameter	Lab	Normality p-value		Wilcoxon p-value
		Pre	Post	
Temperature	Lab 1	0.408	0.001	0.068
Temperature	Lab 2	0.488	0.316	0.066
Temperature	Lab 3	0.272	0.512	0.144
Humidity	Lab 1	0.041	0.024	0.068
Humidity	Lab 2	0.145	0.734	0.068
Humidity	Lab 3	0.858	0.348	1.000
Microbial Load	Lab 1	0.224	0.850	0.715
Microbial Load	Lab 2	0.024	0.195	0.715
Microbial Load	Lab 3	0.369	0.224	0.655

Table 4. The result of the Spearman test

Model	R	R^2	*p*
1	0.293[a]	0.086	0.773

The Spearman correlation analysis showed no significant relationship between airborne microbial loads and the environmental parameters measured (temperature and relative humidity), as indicated by a non-significant p-value ($p=0.773$). The correlation coefficient ($R=0.293$) was low, and the coefficient of determination ($R^2=0.086$) suggested that only 8.6% of the variation in microbial loads could be explained by the environmental variables. This indicates that the environmental parameters measured in this study did not meaningfully influence airborne microbial concentrations.

DISCUSSION

Airborne microbial load

The ongoing investigation has revealed that the airborne microbial loads in the three microbiology laboratories were within the WHO and Indonesian Ministry of Health thresholds, suggesting adherence to biosafety guidelines. Sampling was undertaken at times when the laboratories were not in use for practical classes, thus minimizing human activity and the potential generation of airborne particles. Interviews with laboratory personnel revealed that routine cleaning occurred twice daily, which likely contributed to maintaining low microbial counts and stable indoor air quality throughout the sampling period. Our findings are in line with prior research conducted in West Kalimantan, which reported an average airborne bacterial count of 52.08 CFU/m^3 in the microscopic laboratory, below the minimum level established by the Ministry of Health. This conclusion is likely influenced by the absence of laboratory practicum activities. In addition, environmental factors within the laboratory are not the only determinants of microorganism distribution; human-related factors and activities inside the laboratory can also play a significant role (Atirah et al., 2023). In Malaysian laboratories, a comparable trend was observed, characterized by low airborne microbial loads due to rigorous laboratory cleaning and biosafety standards (Dalee et al., 2016; Hazrin et al., 2015). Likewise, a separate investigation in hospital laboratories observed microbial counts ranging from 9.5 to 199 CFU/m^3, which remained below acceptable thresholds, attributing the findings to regulated airflow (ventilation) and regular cleaning protocols (Emuren & Ordinioha, 2016). Conversely, studies in Nigeria reported higher contamination levels, up to 33×10^2 CFU/m^3, attributed to high occupancy and inadequate ventilation systems (Idemudia et al., 2022). These comparisons suggest that controlled access and

good housekeeping in the present study's laboratories contributed significantly to maintaining microbial levels within safe limits, aligning with global findings that prioritize basic hygiene as a cornerstone of biosafety in academic laboratories.

The Wilcoxon signed-rank test revealed no significant differences between before and after ventilation conditions. However, the descriptive data in Table 1 revealed variations in microbial loads and environmental parameters after ventilation, suggesting that the type of ventilation system influenced indoor air quality. The type of ventilation emerged as an important factor influencing microbial loads across the laboratories. The naturally ventilated laboratory (laboratory 3) consistently exhibited higher total plate counts than laboratories equipped with mechanical air conditioning, although all counts remained below both WHO and national standards (Chawla et al., 2023; Kim et al., 2018; Kementerian Kesehatan Republik Indonesia, 2016; 2023). In Malaysian schools, the same results were obtained: naturally ventilated rooms recorded higher bacterial loads than mechanically ventilated settings (Wamil et al., 2024). Additionally, mechanically ventilated operating rooms with regulated airflow and fewer personnel movements have been shown to have significantly lower microbial levels (Fu Shaw et al., 2018). Indoor rooms with natural ventilation tend to have higher airborne microbial loads because outdoor air enters without filtration, allowing dust, spores, and environmental bacteria to disperse freely into the room (Chawla et al., 2023; Mazlan et al., 2020). In naturally ventilated spaces, airflow patterns are unstable and strongly influenced by wind direction and weather, which increases the resuspension of particles and microbial aerosols from surrounding environments (Yogeswaran et al., 2023). In contrast, mechanical ventilation systems regulate airflow and humidity while providing filtration, resulting in significantly lower microbial concentrations (Dai et al., 2021). High humidity in tropical regions further supports microbial survival, making naturally ventilated rooms more susceptible to increased airborne microbial loads (Jabeen et al., 2023).

Based on observation, no biosafety level in laboratory 3 represents an additional vulnerability, as facilities without standardized biosafety protocols may be more prone to fluctuations in air quality and potential risks during intensive student use. Supporting evidence from assessments in Sudan and the Philippines highlighted that inconsistent decontamination and lack of ISO 15189 compliance can undermine biosafety in academic laboratories despite low baseline microbial levels (Alfy et al., 2022; Wamil et al., 2024). These findings further provide the foundation for formulating practical recommendations in conclusion, particularly regarding ventilation management and the availability of biosafety levels in university laboratories.

Environmental condition

Temperature

Before the ventilation systems were turned on, the average indoor temperature in the three labs was around 30°C. All measurements exceed the standard limit values by the Indonesian Ministry of Health 22–26°C (Kementerian Kesehatan Republik Indonesia, 2016; 2023). Although statistical analysis indicated no significant differences between before and after ventilation conditions, the temperatures dropped to 26–29°C once ventilation was turned on. However, only laboratory 1 met the recommended thermal comfort range. A prior study conducted in Malaysian university laboratories found temperature ranges of 22.7–24.4°C, which matched comfort requirements (Hussin et al., 2014). The increased temperatures after ventilation activation were most likely due to variations in ventilation types (natural and mechanical), the amount of ventilation, and the laboratory position. Detailed differences are presented that laboratory 1 is located on the ground floor, away from direct sunlight.

In contrast, laboratory 2, which is situated on the third floor and exposed to sunlight on one side, whereas laboratory 3, which is located on the fifth level and exposed to direct sunlight on two sides. Despite the availability of central air conditioning, laboratory 2 had higher post-ventilation temperatures than laboratory 1, most likely due to an insufficient ratio of total cooling capacity to room volume, which was worsened by increased solar heat gain from its higher floor

level. Laboratory 3 had the highest thermal variance, indicating both the absence of mechanical cooling and significant sun exposure.

Previous research has shown that differences between air conditioning capacity and room size reduce cooling efficiency and thermal comfort, particularly in larger laboratory settings with significant heat loads (Chen et al., 2019). According to a study at a University in Nigeria, rooms located on higher floors have significantly higher temperatures than those on lower floors due to greater solar radiation and roof exposure (Mba et al., 2025). Other research found that building envelope orientation and sun exposure have a substantial impact on indoor temperatures, with east- and west-facing orientations exhibiting greater heat accumulation throughout the day (Dewanto, 2019). This data, when taken together, emphasizes that attaining stable thermal conditions in university laboratories requires careful consideration of floor location, building orientation, proportional cooling capacity, and regular system maintenance to ensure both biosafety and occupant comfort.

Relative humidity

A similar trend was observed for humidity, with post-ventilation values decreasing across all laboratories but remaining above the Ministry of Health Republic Indonesia's recommended indoor range of 40–60%. Laboratory 1, benefiting from multiple split AC units, experienced the most significant drop in RH (from 62 % to 46 %), approaching the recommended threshold. At the same time, laboratory 2 maintained moderate humidity reductions due to central AC operation. In contrast, laboratory 3, relying solely on natural ventilation, exhibited minimal change inhumidity, with values remaining consistently above 60%. This pattern is consistent with previous studies in tropical academic and healthcare laboratories, which found that mechanically ventilated rooms demonstrated substantial reductions in humidity.

In contrast, naturally ventilated rooms maintained higher RH levels due to uncontrolled moisture exchange with the outdoor environment (Jabeen et al., 2023). Research conducted in Indonesian academic laboratories similarly demonstrated that elevated humidity correlates with increased fungal counts, emphasizing the importance of maintaining humidity within recommended limits to minimize bioaerosol risks (Pramaningsih et al., 2022). These parallels reinforce the conclusion that ventilation type strongly influences humidity stability in tropical climates.

Significantly, the consistently high humidity observed in laboratory 3 is strongly influenced by the local climate characteristics of South Kalimantan, which is classified as a humid tropical region with high average RH throughout the year. Such climatic conditions prolong the survival and stability of airborne microorganisms—particularly fungal spores and several bacterial groups—making spaces without mechanical dehumidification more vulnerable to higher microbial persistence compared to air-conditioned rooms (Jabeen et al., 2023). This contextual factor helps explain why naturally ventilated rooms in tropical regions often retain higher airborne microbial loads even when ventilation is activated.

From a laboratory management perspective, humidity control is therefore essential not only for biosafety but also for preserving the integrity of moisture-sensitive laboratory materials, including culture media and electronic instrumentation. Regular AC maintenance, proper window sealing, and the use of dehumidifiers in naturally ventilated rooms are recommended to stabilize indoor humidity levels and ensure safe, reliable laboratory operations (Suryantoro, 2023).

Microbial loads, temperature, and relative humidity

The Spearman rank correlation analysis demonstrated no statistically significant association between temperature, relative humidity, and airborne microbial counts in both pre- and post-ventilation conditions. Nevertheless, descriptive trends indicated that higher relative humidity was generally associated with higher microbial loads, whereas higher temperatures were associated with lower counts, particularly before ventilation. This pattern suggests that, despite non-significant findings, environmental parameters still influence microbial presence in practical settings, especially in naturally ventilated spaces like laboratory 3.

Similar observations have been reported in previous studies. Research in Indonesian teaching laboratories found that elevated humidity was positively correlated with fungal growth, particularly *Aspergillus* and *Penicillium* species (Pramaningsih et al., 2022). Likewise, a study in Malaysian laboratories found that combined high temperature and humidity enhanced bacterial concentrations (Yogeswaran et al., 2023), while another study observed no significant correlations in facilities with strict cleaning and airflow control (Hazrin et al., 2015). These mixed findings indicate that environmental impacts on microbial loads are context-dependent and are moderated by ventilation type, maintenance routines, and occupancy patterns. Maintaining both temperature and humidity within the recommended ranges (22–26°C; 40–60% RH) is essential in laboratory settings to ensure microbial safety, preserve the integrity of sensitive equipment, and provide comfort for users. Deviations from these ranges should prompt corrective actions, such as routine cleaning of air conditioning units, installation of dehumidifiers in naturally ventilated rooms, and reinforcement of biosafety training for staff and students to sustain safe and reliable laboratory operations.

LIMITATION OF STUDY

This study has several limitations that should be considered when interpreting the findings. First, the pre- and post-ventilation measurements were repeated only twice, which may limit statistical robustness and the ability to detect subtle variations in airborne microbial loads. Second, sampling was conducted during a single weather condition, and seasonal changes, which are known to influence humidity, temperature, and outdoor microbial, were not assessed. Third, bacterial isolates collected during sampling were not identified to the species level, restricting the analysis to total microbial counts and preventing the evaluation of potential pathogenic organisms. Finally, sampling was performed during non-occupancy periods, meaning that the influence of human activity, a major contributor to indoor bioaerosol generation, was not captured in this study.

CONCLUSION

This project demonstrated that airborne microbial loads and environmental conditions in the three university microbiology laboratories remained below both WHO and Indonesian Ministry of Health standards, despite variations in ventilation type and building position. Laboratory 1, which employed multiple split air-conditioning units and was located on the ground floor, consistently maintained optimal temperature and relative humidity compared to laboratories 2 and 3, which were more exposed to solar heat and relied on less effective or natural ventilation. While statistical analysis revealed no significant correlation among temperature, relative humidity, and microbial counts, descriptive trends indicated that higher humidity tended to coincide with higher microbial loads, underscoring the importance of maintaining environmental parameters within recommended ranges.

Ensuring proper temperature and humidity control is critical not only for minimizing microbial proliferation but also for preserving the performance of sensitive laboratory equipment and maintaining user comfort. Routine air-conditioning maintenance, environmental monitoring, and biosafety training should be integrated into laboratory management policies. Additionally, architectural considerations—such as window shading and strategic placement of laboratories—can further support thermal stability and biosafety goals, particularly in tropical academic settings.

AUTHOR'S DECLARATION

Authors' contributions and responsibilities

FIH: Collected and processed the experimentation, data analysis, and drafted the manuscript; **SNA, SKN**: Edited the manuscript, contributed to the interpretation of the data.

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Availability of data and materials

All data are available from the authors.

Competing interests

The authors declare no competing interests.

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