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Evaluation of Microbiological Quality of Indoor Air and Water in Laboratory Environments at the State University of Maringá

Avaliação da Qualidade Microbiológica do Ar Climatizado e da Água em Ambientes Laboratoriais da Universidade Estadual de Maringá

Etyene Maria Moreira Custódio: Universidade Estadual de Maringá. PR, Brasil.

Emilly Brito Ferreira: Universidade Estadual de Maringá. PR, Brasil.

Jean Lopes da Silva: Universidade Estadual de Maringá. PR, Brasil.

Benício Alves de Abreu Filho: Universidade Estadual de Maringá. PR, Brasil.

Fabrícia Gimenes: Universidade Estadual de Maringá. PR, Brasil. E-mail: fgimenes@uem.br

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Abstract

The microbiological quality of air and water in laboratory environments is essential for biosafety, occupational health, and the reliability of analytical results. The increasing use of air-conditioned environments favors the dispersion of microbiological contaminants, especially fungi and bacteria, in addition to enhancing risks associated with water used in laboratories. This study aimed to evaluate, in an integrated manner, the microbiological quality of conditioned air and water in laboratories located in blocks: I90, J90, K10, and T20, belonging to the Departments of Clinical Analysis and Biomedicine and Basic Health Sciences at the State University of Maringá. Air sampling was performed using the impaction method, with population quantification of bacteria and fungi, followed by morphological analysis and Gram staining for bacterial classification. Water samples were analyzed using the chromogenic substrate method for the detection of total coliforms (TC) and *Escherichia coli,* with subsequent biochemical identification of isolates positive for TC. The results indicated that airborne bacterial counts remained within regulatory limits, while most environments showed fungal growth above the maximum recommended value (≤ 750 CFU/m³), with confluent growth. A predominance of Gram-positive cocci was observed in the air, followed by Gram-negative and Gram-positive bacilli. In the microbiological analysis of water, two samples showed contamination by total coliforms, with the identification of *Klebsiella pneumoniae* and *Serratia liquefaciens*. The results highlight the need for preventive maintenance of air-conditioning systems and periodic water monitoring, thereby establishing the continuous adoption of biosafety measures in laboratory environments.

Keywords: Microorganisms. Microbiological Analysis. Quality Control. Air-conditioned Environment.

Resumo

A qualidade microbiológica do ar e da água em ambientes laboratoriais é essencial para a biossegurança, saúde ocupacional e confiabilidade dos resultados analíticos. O uso crescente de ambientes climatizados favorece a dispersão de contaminantes microbiológicos, especialmente fungos e bactérias, além de potencializar riscos associados à água utilizada em laboratórios. Este estudo teve como objetivo avaliar, de forma integrada, a qualidade microbiológica do ar climatizado e da água em laboratórios dos blocos I90, J90, K10 e T20, pertencentes aos Departamentos de Análises Clínicas e Biomedicina e de Ciências Básicas da Saúde da Universidade Estadual de Maringá. A amostragem do ar foi realizada pelo método de impactação, com quantificação populacional de bactérias e fungos, seguida da análise morfológica e coloração de Gram para classificação bacteriana. As amostras de água foram analisadas pelo método do substrato cromogênico para detecção de coliformes totais (CT) e *Escherichia coli,* com posterior identificação bioquímica dos isolados positivos para CT. Os resultados indicaram que as contagens bacterianas do ar permaneceram dentro dos limites normativos, enquanto a maioria dos ambientes apresentou crescimento fúngico acima do valor máximo recomendado (≤ 750 UFC/m³), com crescimento confluente. Observou-se predominância de cocos Gram-positivos no ar, seguidos por bacilos Gram-negativos e Gram-positivos. Nas análises microbiológicas da água, verificou-se que duas das amostras apresentaram contaminação por CT, sendo identificadas as bactérias *Klebsiella pneumoniae* e *Serratia liquefaciens*. Os resultados evidenciam a necessidade de manutenção preventiva dos sistemas de climatização e do monitoramento periódico da água, estabelecendo, portanto, a adoção contínua de medidas de biossegurança em ambientes laboratoriais.

Palavras-chave: Microrganismos. Análise Microbiológica. Controle de Qualidade. Ambiente Climatizado.

1 Introduction

The microbiological quality of air and water in laboratory environments is a determining factor for both occupational health and safety and for the reliability of analyses and products generated within these settings (Kauch; Brągoszewska; Mainka, 2025; Sbibih *et al.*, 2023). The process of urbanization and social development has intensified human permanence in indoor, air-conditioned, and highly occupied environments, progressively replacing outdoor work with activities performed in enclosed spaces (Chamseddine; Elzein and Hassan, 2025; Nie *et al.*, 2025). This phenomenon, associated with the intensification of industrial activities and population growth, has contributed to increased exposure to microbiological contaminants present in the air and on surfaces, in addition to compromising the quality of water used in laboratory and industrial processes (WHO, 2008; Chawla *et al.*, 2023; Kamruzzaman *et al.*, 2026).

In air-conditioned environments, although air does not constitute a natural medium for microbial growth, it serves as an important vehicle for the dispersing of solid particles and droplets containing viable microorganisms, including bacteria, fungi, and viruses (Sénéchal *et al.*, 2015). Air contamination in laboratory settings may compromise biosafety, interfere with analytical results, and affect the reliability of products, especially those intended for direct contact with food (Masotti *et al.*, 2019; Oliveira; Tiwari; Duffy, 2020). These bioaerosols may also cause several adverse health effects, ranging from irritation and allergic reactions to severe respiratory infections (Cox *et al.*, 2019; Yoo *et al.*, 2017). Factors such as inadequate ventilation, high relative humidity, temperature, and the number of occupants may directly influence the microbial composition of indoor air, whereas improperly sanitized air-conditioning systems promote the accumulation of microorganisms in ducts, filters, and moist surfaces, potentially leading to biofilm formation and intensifying the dissemination of contaminants (Bakker, 2020).

According to the World Health Organization (WHO), indoor air pollution constitutes a significant risk factor to human health, responsible for approximately 6.7 million deaths annually worldwide, particularly due to infectious diseases, underscoring its substantial impact (WHO, 2024). In Brazil, there is no federal legislation establishing mandatory, specific legal limits on the maximum concentration of microorganisms in indoor air. Current regulation is based on technical standards, such as Associação Brasileira de Normas Técnicas (ABNT) NBR 17.037:2023, which defines reference values for microbiological air contaminants, establishing limits of up to 750 Colony Forming Units per cubic meter (CFU/m³) for fungi and 500 CFU/m³ for mesophilic bacteria, used as technical parameters for the assessment of air quality in air-conditioned and collective-use environments (ABNT, 2023). These criteria are associated with Law No. 13.589/2018, which establishes the mandatory maintenance and control of indoor air quality through the Maintenance, Operation, and Control Plan (PMOC), without directly establishing specific quantitative values for bioaerosols (Brazil, 2018).

Simultaneously, water is an essential resource for maintaining life and for the functioning of biological, industrial, and laboratory processes. Its microbiological and physicochemical quality depends on the complex interaction between natural and anthropogenic factors and may be compromised by effluent discharge, treatment failures, or inadequate storage conditions (Chinakwe *et al.*, 2022; WHO, 2023). Water contamination represents a serious public health risk, serving as a vehicle for the dissemination of several pathogenic agents (WHO, 2023). It is estimated that approximately 80% of diseases in developing countries are associated with water contamination (WHO, 2023), particularly amoebiasis, giardiasis, typhoid fever, leptospirosis, ascariasis, cholera, and gastroenteritis (Pratap *et al.*, 2023).

The microbiological analysis of water is based on detecting contamination indicators, such as total coliforms (TC) and thermotolerant coliforms, with *Escherichia coli* as the primary bioindicator of fecal pollution (Veras *et al*., 2025). Brazilian legislation requires the absence of TC and *E. coli* in 100 mL of water for it to be considered potable, while standards such as CONAMA Resolutions No. 357/2005 and No. 396/2008 establish compatible parameters for different uses, including laboratory applications (Brasil, 2021).

Thus, the integrated assessment of the microbiological quality of air-conditioned air and water constitutes an essential strategy for biosafety and environmental quality control, enabling the identification of potential sources of cross-contamination, the prevention of health risks, and the assurance of the reliability of analytical results. In universities and health-related research centers, the risk of contamination is even greater, mainly due to the high volume of people, the handling of microorganisms, and the high concentration of activities in enclosed spaces, underscoring the importance of monitoring and properly maintaining facilities (Chawla *et al*., 2023). Despite this, studies investigating the integrated microbiological quality of air and water in university laboratories remain scarce.

In this context, the present study aimed to evaluate, in an integrated manner, the microbiological quality of indoor air-conditioned air and water in laboratory and common-use environments of blocks I90, J90, K10, and T20, belonging to the Departments of Clinical Analysis and Biomedicine (DAB) and Basic Health Sciences (DBS) of the State University of Maringá (UEM) (Maringá, Paraná, Brazil). These buildings house health-related undergraduate programs, as well as the Teaching and Research Laboratory in Clinical Analysis (LEPAC), which is characterized by the intense flow of students, staff, and public service activities. Overall, this study contributes to identifying potential health risks and establishing biosafety measures to reduce exposure to contaminants and ensure adequate conditions in collective environments for teaching, research, and professional training.

2 Material and Methods

The study was conducted at four strategic locations situated at the State University of Maringá. The geographic coordinates are as follows: Block I90 (23°24'09"S, 51°56'24"W), Block J90 (23°24'09"S, 51°56'26"W), LEPAC – Block K10 (23°24'07"S, 51°56'27"W), and Block T20 (23°24'06"S, 51°56'28"W). These locations belong to the DAB and DBS departments. They were selected due to the high student volume and the continuous use of laboratories for teaching, research, and activities involving the handling of microorganisms. In addition, Block K10 houses the LEPAC.

This center provides services to the population of the Northwestern macro-region of Parana State. It covers 113 municipalities and performs highly complex laboratory examinations. The spatial distribution of the sampling sites is shown in Figure 1.

Figure 1 - Spatial distribution of the sampling sites on the campus of the UEM, located in blocks I90, J90, K10 (LEPAC) and T20

Source: the authors.

2.1 Microbiological analysis of air

2.1.1 Sampling of air-conditioned air

To evaluate the microbiological quality of air, air-conditioned laboratories located in the previously mentioned buildings were selected (Table 1). Sampling was carried out monthly over three consecutive months: May, June, and July 2025.

Table 1 - Sampling sites for air-conditioned air

Block	Laboratory
I90	Microbiology Laboratory (Room 115), Toxicology Laboratory (Room 207 F), and Pathology Laboratory (Room 002).
J90	Clinical Cytology Laboratory (Room 011).
T20	Water, Environmental, and Food Microbiology Laboratory (Room 314), Medical Bacteriology Laboratory (Room 213), and Immunology Laboratory (reception area).
K10	LEPAC (reception area and special collection room).

Source: research data.

Air-conditioned air sampling was performed using impaction with the Air Ideal® 3P equipment (BioMérieux, France). The sampler was positioned in the central region of the environments, at a height of 1.5 m above the floor, and operated at a flow rate of 100 L/min for 3 minutes. This protocol constitutes an adaptation of ANVISA Resolution RE No. 9 (Brazil, 2003), which recommends a flow rate of 28.3 L/min. The adjustment enabled the collection of a comparable air volume (300 L) over a shorter time interval.

Plate Count Agar (PCA) was used for the enumeration of heterotrophic bacteria, and 2% Sabouraud Dextrose Agar was used for fungal (molds and yeasts) counts. In each environment, two to three sampling points were established, all performed in duplicate. In addition, duplicate samples were collected from the outdoor environment of each building block and were used as controls for fungal growth analysis.

2.1.2 Laboratory analysis

After sampling, the samples were immediately transported to the Water, Environmental, and Food Microbiology Laboratory (LAGUA-UEM) for incubation and analysis. Samples cultured on 2% Sabouraud Dextrose Agar were incubated at 28 °C and analyzed at 24 h intervals, from the first to the seventh day of incubation (168 h). Quantification was expressed as CFU/m^3 and compared with the limits established by ABNT NBR 17.037:2023.

Samples cultured on PCA were incubated at 35 °C and evaluated after 24 h and 48 h. Colonies were selected based on macroscopic morphological characteristics, such as color, shape, and consistency. Each morphotype was isolated only once; colonies with similar morphological patterns, even when originating from distinct samplings, were compared with previous records and not selected again. The isolates were transferred to sterile saline solution, streaked onto Tryptic Soy Agar (TSA), and incubated at 35 °C for 24 h. Gram staining was performed to evaluate the morphological and cultural characteristics of the isolates.

2.2 Microbiological Analysis of Water

2.2.1 Water sampling

Water sampling was conducted different indoor environments selected based on frequency of use (Table 2). Sampling was carried out monthly over three consecutive months (May, June, and July 2025), using sterile bottles manufactured by Idexx containing 10 mg of sodium thiosulfate for neutralization residual chlorine.

A volume of 100 mL was collected for each sample, which was refrigeration and transported to the laboratory within 2 hours of collection (APHA, 2022). Prior to collection, faucets were disinfected with 70% alcohol and then opened or pressure-activated (for drinking fountains) for 1 minute to minimize contamination from contaminants at the sampling point.

Table 2 - Water sampling sites

Block	Room/Sampling Site
I90	Hallway drinking fountain (ground floor); Microbiology Laboratory (3rd floor, Room 117, faucet for material washing); and hallway drinking fountain (3rd floor).
J90	Hallway drinking fountain and Clinical Cytology Laboratory (Room 011, handwashing faucet).
T20	Hallway drinking fountain (3rd floor); pantry faucet (2nd floor, Room 2); and Immunology Laboratory (1st floor, hallway drinking fountain).
K10	LEPAC (reception area drinking fountain and faucet in the special collection room).

Source: research data.

2.2.2 Laboratory analysis

The samples were sent to LAGUA-UEM for the presence/absence test of TC and *E. coli*. The chromogenic substrate technique was used with the commercial Colisure® kit (IDEXX Laboratories Inc., USA). This method is based on the detection of enzymatic activities, such as β-galactosidase for TC and β-glucuronidase for *E. coli*, providing a rapid, sensitive, and reliable assessment of microbiological contamination. The contents of one reagent vial were added to 100 mL of each water sample, and the mixture was incubated at 35 ± 0.5 °C for 24 h. The result was considered positive for TC when the sample color changed from yellow to magenta, whereas the presence of *E. coli* was evidenced by blue fluorescence under UV light exposure (365 nm) (APHA, 2022).

Subsequently, TC-positive samples were isolated and subjected to Gram staining and oxidase testing. After confirmation of Gram-negative bacilli and the absence of the oxidase enzyme, specific biochemical identification was performed using the commercial Bactray system (Laborclin, Brazil) through kits I and II. Final analysis of the biochemical profiles was carried out using the Bactray Web software (Laborclin, 2024; Crispim *et al*., 2026).

2.3 Statistical analysis

All determinations were performed in triplicate, and the results are expressed as mean ± standard deviation. Data were subjected to analysis of variance (ANOVA), followed by Tukey's test ($p < 0.05$) for comparison of means. Statistical analyses were performed using Statistica® software version 10.0 (StatSoft Inc.).

2.4 Ethics statement

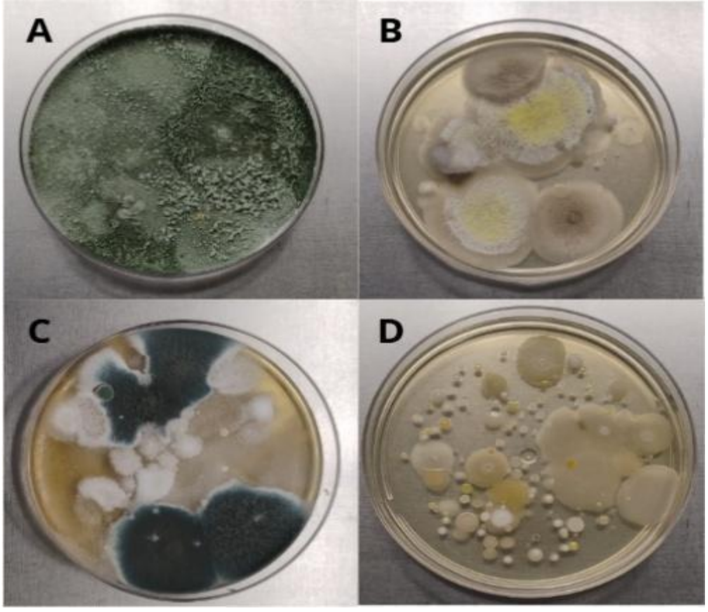
This study did not involve human subjects, animals, or identifiable biological materials and, therefore, did not require approval by a Research Ethics Committee, in accordance with applicable regulations. The study was conducted exclusively in laboratory settings, and prior authorization was obtained from the Department of Basic Health Sciences institutional coordination at the State University of Maringá.

3 Results and Discussion

3.1 Microbiological analysis of air

According to ANVISA Resolution No. 9 (Brazil, 2003), the reading, or counting of fungal colonies must be performed after seven days of incubation. However, studies have demonstrated that, within shorter periods, mycelial growth may cover the surface of the culture medium, rendering individual colony counting unfeasible. Therefore, daily monitoring of the plates until growth stabilization is recommended, which generally occurs within 7 days (Bao *et al.*, 2021; Paiva *et al.*, 2023). In the present study, intense growth of fungal and bacterial colonies was observed, as illustrated in Figure 2.

Figure 2 - Fungal and bacterial growth after a 48-hour incubation period. A and B: Microbiology Laboratory on Sabouraud and PCA media, respectively. C and D: Clinical Cytology Laboratory, located in Block J90, on Sabouraud and PCA media, respectively



Source: the authors.

Regarding the counting of fungal colonies (molds and yeasts), Associação Brasileira de Normas Técnicas NBR 17.037:2023 establishes that, in air-conditioned indoor environments, the maximum allowable concentration is ≤ 750 CFU/m³ (ABNT, 2023). It was observed that most of the evaluated environments exhibited confluent fungal growth after only 48 hours of incubation, rendering accurate colony quantification unfeasible. This profile suggests a high fungal bioaerosol load in the analyzed environments and may reflect a combination of factors favorable to the dispersion and persistence of these microorganisms, including high human traffic, insufficient air renewal, and potential moisture accumulation in air-conditioning systems. In laboratory environments, especially those intended for teaching and microbiological manipulation, such conditions may favor the continuous suspension of contaminated particles and the establishment of microbial reservoirs on surfaces and ventilation ducts. In addition to its impact on environmental quality, the elevated fungal load is also of occupational and analytical relevance, given the potential of these microorganisms to trigger allergic reactions, cause opportunistic infections, and interfere with sensitive laboratory procedures.

The exception was the third collection performed at Block T20 – Medical Bacteriology Laboratory (Room 213), which recorded a count of 48.5 CFU/m³, within compliance with the regulatory standard. The remaining environments showed values above the established limit, suggesting a greater risk of occupational exposure to bioaerosols potentially associated with allergic manifestations, respiratory disorders, and opportunistic infections. Furthermore, colony counts from the control plates used for fungal growth also showed confluent growth in all collections. Additionally, the aforementioned standard recommends that the ratio of fungal concentrations between indoor and outdoor environments (I/O) be ≤ 1.5, which is used as an auxiliary criterion for assessing the microbiological quality of air (ABNT, 2023).

However, this index could not be determined because most fungal samples exhibited confluent growth, preventing accurate quantification.

Exposure to bioaerosols, including fungi, has been widely associated with adverse effects on human health (Cox *et al*., 2019). Airborne fungi are of considerable clinical importance, as they are associated with allergic and infectious processes. Among the most frequent clinical manifestations are rhinitis, allergic asthma, extrinsic allergic alveolitis, non-invasive allergic sinusitis, and pulmonary mycoses, many of which are related to occupational environments (Yoo *et al*., 2027; Ramírez *et al*., 2025).

Table 3 presents the bacterial colony counts (CFU/m³) at the different sampling points after a 48-hour incubation period, along with the mean and standard deviation of the three collections.

Table 3 - Bacterial colony counts in air-conditioned air after 48 hours of incubation

Analyzed Locations	**May**	**June**	**July**
	1º Collection	**2º Collection**	**3º Collection**
T20 - LAGUA - UEM	155Cb ± 2.00	233.5Ba ± 36.5	205Ca ± 15.00
T20 - Medical Bacteriology	138Ca ± 15.00	188Cb ± 25.00	48.5Dc ± 18.75
T20 – Immunology	240Ba ± 20.00	148Cc ± 45.00	216.5Cb ± 6.75
Microbiology	100Cb ± 7.00	83.5Db ± 3.5	263.5Ca ± 54.10
Toxicology	185Ca ± 53.00	47Dc ± 1.00	93.5Db ± 13.50
Pathology	185Cb ± 2.00	258.5Ba ± 31.50	113Dc ± 10.00

Clinical Cytology	348.5Aa ± 50.00	103.5Db ± 23.50	78.5Db ± 22.30
LEPAC - Special Collection Room	178Cb ± 15.00	398.5Aa ± 70.00	340Ba ± 12.00
LEPAC - Reception	170Cb ± 20.00	410Aa ± 100.00	433.5Aa ± 33.50

*Means followed by the same lowercase letter within the column do not differ significantly according to Tukey's test ($p < 0.05$). Means followed by different uppercase letters within the row differ significantly according to Tukey's test ($p < 0.05$).

Source: research data.

Although all bacterial counts remained below the maximum limit established by the Associação Brasileira de Normas Técnicas NBR 17.037:2023 (ABNT, 2023), indicating microbiological compliance with bacteria in the evaluated environments, statistically significant differences were observed among the locations and months ($p < 0.05$).

In comparison locations within each collection period (uppercase letters), the LEPAC units, especially the Reception Area and the Special Collection Room, had the highest bacterial counts during the second and third collections, which differed statistically from the other environments. In contrast, the Toxicology and Medical Bacteriology laboratories generally exhibited the lowest mean values, grouping among the sectors with the lowest contamination levels.

In the comparison among collections within each location (lowercase letters), significant differences were also identified. The Clinical Cytology Laboratory exhibited the highest count during the first collection, followed by a marked reduction in the subsequent months.

Conversely, the LEPAC areas showed a progressive increase throughout the collections, indicating intensification of the microbial load over the study period. Other environments, such as the Microbiology and Pathology laboratories, also displayed fluctuations among the evaluated months.

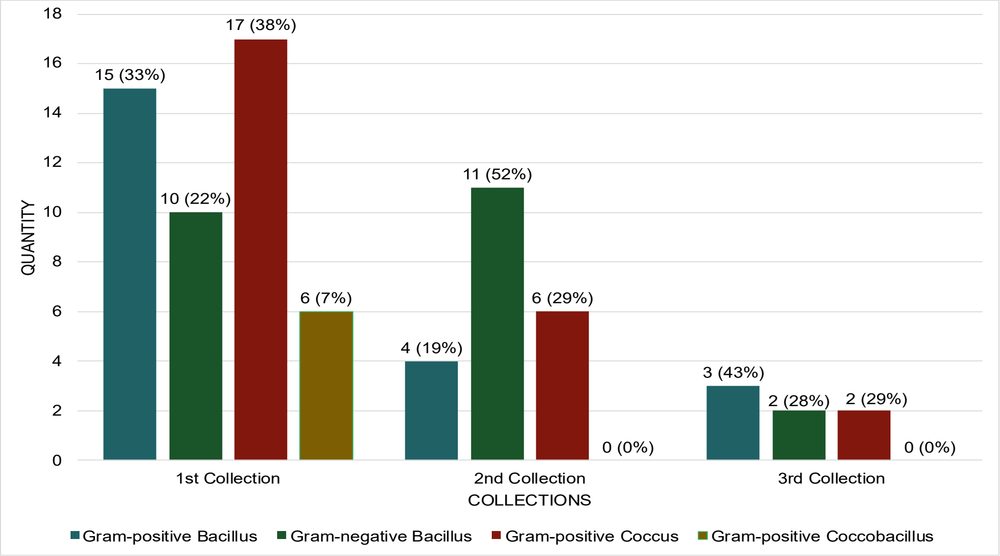
The variability observed across environments suggests that operational characteristics and occupancy dynamics strongly influence bacterial load in air-conditioned air. The highest values recorded in the LEPAC areas, particularly in the Reception Area and Special Collection Room, are likely related to the intense daily flow of individuals, increased movement of biological materials, and frequent door opening, all of which favor particle suspension and the dissemination of bioaerosols.

In contrast, environments such as the Toxicology and Medical Bacteriology laboratories presented lower bacterial counts, possibly due to more restricted access, lower circulation of individuals, and stricter control of biosafety practices.

Furthermore, temporal fluctuations across the collections indicate that seasonal environmental factors, including temperature, relative humidity, and air-conditioning efficiency, may directly influence airborne microbial dynamics.

In addition to this quantitative analysis, morphological characterization and Gram staining of the isolated bacteria were performed, colonies exhibiting the same morphological pattern were analyzed only once. The microorganisms were classified as Gram-positive bacilli, Gram-negative bacilli, Gram-positive cocci, and Gram-positive coccobacilli (Figure 3).

Figure 3 - Morphological characterization and Gram staining of bacteria in each collection performed



Source: the authors.

Across the three collections, 73 distinct bacterial morphotypes were identified. A predominance of Gram-positive cocci was observed, comprising 25 samples (34.3%), followed by 23 samples of Gram-negative bacilli (31.5%), 22 samples of Gram-positive bacilli (30.1%), and 3 samples of Gram-positive coccobacilli (4.1%). It should be emphasized, as a limitation of the study, that the microbiological identification of airborne microorganisms, performed based on morphological characteristics and Gram staining, does not allow taxonomic determination at the genus and species levels, thereby limiting more specific clinical inferences, although this does not compromise the assessment of environmental microbiological risk.

The high frequency of Gram-positive cocci, often represented by *Staphylococcus* spp. and *Micrococcus* spp., is consistent with environments characterized by intense human circulation, since these microorganisms are part of the skin and respiratory tract microbiota and may act as opportunistic pathogens, especially in immunocompromised individuals (Gómez-Sánchez *et al.*, 2025; Lee *et al.*, 2024). The presence of Gram-positive bacilli, in turn, may be associated with *Bacillus* spp., which are known for their resistance to adverse environmental conditions, thereby favoring their persistence in the air and on surfaces (Jin *et al.*, 2025). Additionally, the detection of Gram-negative bacilli warrants particular attention, as this group includes microorganisms with greater virulence potential and antimicrobial resistance, such as species in the genera *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacter* spp. The presence of these microorganisms in the air indicates a potential risk for respiratory infections and other clinical complications, especially in hospital, laboratory, or high-traffic environments, reinforcing the need for continuous microbiological monitoring and proper maintenance of air-conditioning systems (Nikaeen; Shamsizadeh; Mirhoseini, 2018; Stockwell *et al.*, 2019).

The detection of this bacterial group in healthcare-related university environments deserves special attention, as environmental Gram-negative species may act as reservoirs of antimicrobial resistance genes and exhibit a high capacity for persistence on moist surfaces and within air-conditioning systems. Therefore, even at concentrations below regulatory limits, the presence of these microorganisms may represent a potential risk to immunosuppressed individuals and to cross-contamination in laboratory environments (Kanamori *et al.*, 2023).

3.2 Microbiological analysis of water

The microbiological analysis of water indicated compliance at most sampling points throughout the study period. Positive results for total coliforms were observed only in samples 0012 (Immunology Laboratory – 1st floor drinking fountain) and 0015 (Microbiology Laboratory – sink faucet, Room 117, 3º floor), both corresponding to the second collection, characterizing isolated contamination events.

Although most samples showed microbiological compliance standards, occasional detection of total coliforms in laboratory environments should not be neglected. Even isolated contamination episodes indicate vulnerability within the internal water distribution system and suggest possible localized failures in cleaning, maintenance, or water stagnation. In university environments dedicated to teaching and health research, such findings are particularly relevant, given that water is used in laboratory activities, for material sanitation, and for human consumption, and may serve as an indirect source of cross-contamination (Yiek *et al.*, 2021).

Isolated contamination events at specific points within the internal distribution system may be associated with factors such as biofilm accumulation in faucets and drinking fountains, localized failures in cleaning and equipment maintenance, intermittent use of the hydraulic system, or periods of water stagnation, which favor microbial adhesion and proliferation (Oliver; Paula; Veiga, 2021). Such isolated episodes are common in locations with high user traffic and greater handling at consumption points.

For microorganism identification, the two positive samples were isolated and characterized microbiologically. Gram staining revealed Gram-negative bacilli, while the oxidase test yielded negative results, corroborating the initial identification of coliforms and indicating that the isolates belonged to the order *Enterobacterales*. This identification is relevant because this group includes microorganisms of major clinical and sanitary importance, frequently associated with environmental contamination and opportunistic infections, including urinary tract infections, gastrointestinal infections, and healthcare-associated infections (HAIs) (Dokuta *et al.*, 2025: Perera *et al.*, 2022). Based on the oxidase-negative profile, detailed biochemical identification was subsequently performed (Table 4).

Table 4 - Biochemical identification of Gram-negative bacilli isolated from the total coliform (TC) test

Samples	**ONG**	**ADH**	**LDC**	**ODC**	**H_2S**	**URE**	**VPT**	**PDT**	**IND**	**CIT**	**MAL**	**RAM**	**ADO**	**SAL**	**ARA**	**INO**	**SOR**	**SAC**	**MAN**	**RAF**	**Isolates**
0012	+	-	+	-	-	+	+	-	-	+	-	-	-	-	+	-	+	+	+	+	*Serratia liquefaciens*
0015	+	-	+	-	-	+	+	-	-	+	+	+	+	+	+	+	-	+	+	+	*Klebsiella pneumoniae* subsp. *pneumoniae*

OXI: Oxidase; ONG: β-galactosidase production; ADH: Arginine dihydrolase activity; LDC: Lysine decarboxylase activity; ODC: Ornithine decarboxylase activity; H_2S: Hydrogen sulfide production; URE: Urea degradation; VPT: Voges–Proskauer test; PDT: Phenylalanine deaminase production; IND: Indole production; CIT: Citrate utilization; MAL: Malonate utilization; RAM: Acid production from rhamnose; ADO: Acid production from adonitol; SAL: Acid production from salicin; ARA: Acid production from arabinose; INO: Acid production from inositol; SOR: Acid production from sorbitol; SAC: Acid production from sucrose; MAN: Acid production from mannitol; RAF: Acid production from raffinose.
Source: research data.

The biochemical tests allowed the classification of the isolates from sample 0015 as *Klebsiella pneumoniae* subsp. *pneumoniae* and those from sample 0012 as *Serratia liquefaciens*, microorganisms recognized for their pathogenic potential and clinical importance. *K. pneumoniae* is an opportunistic pathogen capable of colonizing mucosal surfaces and disseminating to other tissues, potentially causing severe and life-threatening conditions (Abbas, 2024). Virulent strains of this species frequently exhibit resistance to multiple antimicrobials and are associated with several clinical manifestations (Douradinha, 2023), including pneumonia (Li *et al.*, 2024), bacteremia (Hafiz *et al.*, 2023; Monteiro *et al.*, 2025), and urinary tract infections (Monteiro *et al.*, 2025). These infections are characterized by high morbidity and mortality, in addition to their potential for metastatic dissemination (Abbas, 2024). *S. liquefaciens*, in turn, has been described as an opportunistic bacterium associated with nosocomial infections, mainly affecting immunocompromised patients or individuals using invasive devices, such as catheters and mechanical ventilation (Monardo *et al.*, 2025). Both microorganisms are noteworthy for their ability to cause severe infections, particularly after gaining access to the bloodstream, urinary tract, or respiratory system (Pérez-Viso *et al.*, 2024).

The identification of these bacteria at water consumption and usage points reinforces the importance of continuous microbiological monitoring of the internal water distribution system, especially due to the ability of these microorganisms to persist on moist surfaces and form biofilms, structures associated with protection against sanitizing agents and the maintenance of microbial reservoirs in water systems (Oliver, Paula, and Veiga, 2021; Pérez-Viso *et al*., 2024). In healthcare-related university environments, this condition becomes even more relevant, given the high volume of individuals and the potential for cross-contamination during laboratory and healthcare activities.

Despite the relevance of the results, some limitations should be considered, including the absence of molecular identification of the isolates and the failure to evaluate environmental physicochemical parameters potentially associated with the observed microbial dynamics. Nevertheless, the findings highlight the importance of environmental microbiological monitoring and preventive maintenance of air-conditioning and water supply systems in healthcare institutions, given their impacts on biosafety, occupational health, and the reliability of laboratory activities.

4 Conclusion

The results of this study highlight the importance of continuous monitoring of the microbiological quality of air-conditioned air and water in laboratory environments at the UEM. Air evaluation demonstrated that bacterial counts were within the limits established by Associação Brasileira de Normas Técnicas NBR 17.037:2023. However, elevated fungal growth, with confluent formation, was observed, rendering accurate counting unfeasible and indicating non-compliance with regulatory parameters. Microbiological characterization revealed a predominance of Gram-positive cocci, followed by Gram-negative and Gram-positive bacilli, with the detection of Gram-negative bacilli being particularly relevant due to their greater virulence potential and antimicrobial resistance.

Although most analyzed water samples compliance with current standards, two samples tested positive for total coliforms and were identified as *Klebsiella pneumoniae* subsp. *pneumoniae* and *Serratia liquefaciens*. These findings demonstrate the vulnerability of the internal distribution network and highlight potential health risks to users and workers.

Overall, the study demonstrates that, even in institutional environments that follow biosafety protocols, preventive maintenance of air-conditioning systems and water quality control, associated with continuous environmental monitoring, constitute fundamental measures to minimize the risks of microbiological dissemination. These preventive actions promote occupational health, user safety, and the reliability of teaching, research, and healthcare-related activities, while also supporting improvements in environmental management practices and the development of future investigations within the institution.

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