

**Universidade Federal de Uberlândia
Instituto de Ciências Biomédicas
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**BACTERIAL BIOAEROSOL AND ENVIRONMENTAL VARIABLES ASSESSMENT
OF CRITICAL HOSPITALIZATION UNITS OF A TERTIARY HOSPITAL**

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Trabalho de conclusão de curso
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Bacterial Bioaerosol and Environmental Variables Assessment of Critical Hospitalization Units of a Tertiary Hospital

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Abstract

Components of bioaerosols can be dangerous for indoor air quality (IAQ) in hospital buildings. Presence of bacteria in those aerosols may lead to hospital-associated infections and an enlarged number of occupational diseases, being harmful to healthcare workers and patients who may have vulnerable immune systems. This study aimed to evaluate bacteriological contamination of bioaerosols and IQA parameters in a tertiary hospital. The study was performed in four specialized hospitalization critical units (Infectious Disease, Clinical Oncology, Burn and Plastic Surgery, Kidney-Transplanted) located at an university tertiary hospital before and during the *SARS-CoV-2* pandemic. Air samples were collected by impaction using the single-stage sampler to quantify, isolate, and identify airborne bacteria. The environmental variables concentration of particulate matter (PM), the concentration of carbon dioxide CO², temperature, and relative humidity were analyzed by appropriate equipment to each sampling. The concentration of airborne bacteria varied from 51.22±8.89 to 264.11±161.36 CFU/m³. Of the thirteen bacterial genera identified, eleven were potentially pathogenic and/or opportunistic. The variables temperature and relative humidity were higher than indicated. We concluded that there is a need to improve IAQ and that the new sanitary parameters established during the *SARS-CoV-2* pandemic influenced the concentration of colony-forming units and the total number of bacteria identified in the two phases of this research. A more comprehensive evaluation is recommended to analyze other influential factors for a complete view of the bioaerosol composition, and possible adoption of IAQ control for each specialized unit studied and other sectors of the hospital.

Key words: bioaerosol, airborne, bacteria, air quality, hospital.

1. Introduction

The definition of bioaerosols is solid, liquid, or gaseous particulate matter (PM) of microbial, plant, or animal origin. It may contain pathogenic or non-pathogenic live or dead bacteria or bacterial endotoxins, fungi, viruses, high molecular weight allergens, pollen, and plant fiber, among other components suspended in a gas (Douwes et al., 2003; Nazaroff, 2016; Soleimani et al., 2016). Indoor exposure to bioaerosol may cause or contribute to human health issues, including infectious diseases, acute toxic effects, and allergies (Douwes et al., 2003; Dehghani et al., 2018).

Indoor bacterial bioaerosols composition and concentration are predominantly produced in the outdoor ambient atmosphere and carried in through windows, doors, and applications of heating, ventilation, and air-conditioning systems in addition to those aerosolized internally. Different human activities are capable to emit airborne bacteria, such as talking, sneezing, coughing, walking, and others. The density of people on the room can also influence the concentration of those particles. Due to the number of different sources, it is arduous to describe the composition type of the bioaerosol and its microbial contamination (Ponsoni & Raddi, 2010; Nazaroff, 2016, Mirskaya & Agranovski, 2018)

Ensuring healthy indoor air quality (IAQ) in hospital buildings is essential to guarantee the well-being of healthcare workers and patients, who may have vulnerable immune systems from damage caused by hazardous components of bioaerosols (Cabo Verde et al., 2015; Soleimani et al., 2016). The presence of bacteria in those aerosols may lead to hospital-associated infections (HAIs) and an enlarged number of occupational diseases. In these facilities, the spread of microbiological contamination occurs mainly through patients, their relatives, healthcare workers, although it also happens through ventilation appliances (King et al., 2013; Chung et al., 2015; Yousefzadeh et al., 2022).

In Brazilian legislation, the National Environment Council (CONAMA) via the Air Quality Index (IQAR) (Resolution n° 491) and the Brazilian Health Regulatory Agency (ANVISA) through the Reference Standards for Indoor Air Quality in Artificially Air-conditioned Environments for Public and Collective Use (Resolution n° 09), regulate only a few components of bioaerosol and IAQ whereas there is no regulation for the presence of bacteria (Brasil, 2003; Brasil, 2018).

Regarding bacterial agents, elements as such endotoxins, present in the outer membrane of Gram-negative bacteria, and peptidoglycan, in the cell wall of Gram-positive bacteria, are capable of inducing inflammatory responses and lead to the development of respiratory diseases (Douwes et al., 2003; Nazaroff, 2016; Robertson et al., 2019). Previous investigations have already identified bacterial contamination of hospital air caused by *Staphylococcus aureus* (Gizaw et al., 2016; Nasiri et al., 2021), *Pseudomonas aeruginosa* (Pereira et al., 2005; Nasiri et al., 2021)

In this study, different environmental variables components of IAQ and bioaerosols were analyzed. The toxicity and clinical impact of the variable particulate matter depend on the pulmonary deposition in the respiratory tract and the complex chemical constitution of those particulate matters. There are three possible deposition sites in the respiratory system depending on the PM diameter: Coarse particles have a median aerodynamic diameter of 10 to 2,5 μm (PM₁₀) and can effortlessly deposit in the upper tracheobronchial tree; fine particles with a median aerodynamic diameter of <2.5 μm (PM_{2.5}); and ultrafine particles (UFP), smaller than 0.1 μm , are capable of penetrating small airway regions such as the alveolar region (Brook et al., 2004; Vincent, 2005; Losacco & Perillo, 2018). Furthermore, published articles by Fiordelise et al. (2017) and Kim et al. (2020) report that PM_{2.5} can be absorbed into the bloodstream through alveolar capillaries and cause lung and systemic inflammation.

Another environmental variable is carbon dioxide (CO₂), which has a consistent relationship established with the number of occupants in a room. Even though it is relatively nontoxic, high concentrations of this gas can provoke heat, smothering sensation, and shortness of breath in the occupants and affect the health and comfort of patients (Ponsoni e Raddi, 2010; Madureira et al., 2015; Cincinelli & Martellini, 2017). ANVISA recommends that the maximum concentration in closed environments stay under 1000 ppm to avoid chemical CO₂ contamination (Brasil, 2003).

Weather conditions, like temperature and humidity, can have a variety of impact on bioaerosol concentrations, due to the ability of these meteorological variations to initiate the dispersion of biological components present in bioaerosols (Jones & Harrison, 2004; Frankel et al., 2012). In addition, exceed the criteria indicated by legislation may cause discomfort, beyond that it contributes to making the environment more favorable to the proliferation of fungi and bacteria (Brasil, 2003; Brasil, 2018). The association of temperature, its impact on human health, and mortality have already been stated in the literature, but its relation to airway

pollution concentration and particulate matter is described by the authors as inconsistent (Basu & Samet, 2002; Roberts, 2004; Ren & Tong, 2006; Guo et al., 2016; Tian et al., 2018).

The objective of this study was to evaluate the indoor air quality of specialized critical hospitalization units from a Brazilian tertiary hospital about the bacteriological parameters, the concentration of carbon dioxide, particulate matter, temperature, and humidity, contributing to the knowledge of the IAQ of those areas and safety of its users.

2. Material and Methods

2.1. Setting

The Clinical Hospital of the Federal University of Uberlandia (HC-UFU/EBSERH) is a public academic tertiary hospital located in Uberlandia, Minas Gerais state, Brazil. The hospital has over 500 beds, and it is reference for urgency, emergency, and middle-high-complexity care, being the only regional public hospital permanently open of that category.

The sampling of this research occurred in four specialized critical hospitalization units from the HC-UFU: Infectious Disease, Clinical Oncology, Burn and Plastic Surgery, and Kidney-Transplanted units.

Sample collection occurs in two phases, during the wet season of the country (October to March). The first sample was carried between November 2019 and February 2020 designated as the pre-pandemic phase, and the second occurred between November 2020 and February 2021, referred as pandemic phase due to the *SARS-CoV-2* pandemic, that started in early 2020.

The samples assessment occurred in triplicates, for every unit, throughout three days during working hours. The number of occupants present within the room at the beginning of the measurement was registered. For each day of the analysis, the evaluation of the air outside the hospital building was performed as a control.

2.2. Bacteriological Evaluation

For the evaluation of aerobic and facultative anaerobic bacteria in each unit, were used a single-stage sampler (MAS-100, Merck KGaA, Germany), which aspirates 500 L of air for 5

minutes onto Petri plates (90 mm), the device was positioned at approximately 1.5 m from the ground. The samples were incubated for 48 hours at 37°C in a bacteriological incubator.

Tryptic Soy Agar (TSA, Difco) was used to measure the colony-forming units of bacteria per cubic meter (CFU/m³) following the conversion table provided by the one-stage sampler manufacturer. The control assessment was realized through the same procedures in the external environment, defined control CFU/m³, and established the indoor air and external air relationship daily (I/Eday).

To identify the type of bacterial contaminants, Mannitol Salt Agar (SMA, Difco) was used to isolate *Staphylococcus* sp, MacConkey Agar (MCA, Difco) for Gram-negative bacteria, Pseudomonas Agar (PA, BD) to isolate *Pseudomonas* sp, and chromID VRE (bioMérieux's) agar for the detection of *E. faecium* and *E. faecalis* with acquired vancomycin resistance (VRE).

The colonies isolated at MSA, MCA, PA, ChormID VRE were submitted to Gram staining and bacterial identification through Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The microbial colonies were suspended in 300 µL distilled water, added 900 µL of 99.5% alcohol and centrifuged at 13,000 rpm for 2 min. Subsequently, the supernatant was discarded, 20 µL of 70% formic acid was added, and the final solution was vortexed. After vortexing, 20 µL of acetonitrile was added and centrifuged for two min at 13,000 rpm. Then, aliquots of the supernatant were analyzed using mass spectrometry (Bruker MALDI Biotyper 4.0). The criteria established for identification were: ≥ 2.0 for species and ≤ 1.7 for the genus (Tarumoto et al., 2016).

2.3. Particulate Matter Concentration

To evaluate the particulate matter concentration was used the environmental monitor TSI Quest EVM-7 (3M™ EVMMR Series, USA) that can measure and log mass concentration particulate was used. The monitor is programmed to aspire particles smaller than 2.5 µm in diameter (PM2.5) over the course of one hour, positioned approximately at 1.5 above floor level. This instrument permits the data analysis of mean, maximum, and minimum concentration data.

2.4. Carbon Dioxide Concentration

For the assessment of CO₂ concentration, the monitor TESTO 535 (BSRIA Instrument Solution, England) was used. The portable sensor was positioned approximately at 1.5 above floor level, the measurement of mean, maximum, and minimum data occurred every 30 minutes during three and a half hours in each unit and equally for the external Control.

2.5. Temperature and Relative Humidity

To measure the temperature and relative humidity a digital thermohygrometer (Valley Great Tools, Australia) was used, the device was positioned 1.5 meters from the floor, one hour after the beginning of the experiment, the momentary temperature and relative humidity in addition to the minimum and maximum temperatures registered during this period was recorded.

2.6. Statistical analysis

To avoid inflation of the degrees of freedom in correlation and regression analyses, the CFU/m³ and CO₂ data (minimum, average and maximum) were reduced to the mean values within each collection data, representing a repetition per day. Such correction also avoids matching error within each day of collection between measurements, although the temporal difference between them can be considered irrelevant (measured in a time interval of 100 to 210 minutes). Independent of this, the regression models take into account the average value in the estimates at each sampling points where there is replication, without prejudice to the estimation of the parameters.

The quantitative variables evaluated are either counts or positive or have null values. Given this, the means of the variables were compared between the four hospitalization units with Generalized Linear Models. The probability distribution function that best fit the data, selected according to Akaike's criterion, was adopted in each variable. The distributions were selected between Poisson, Negative Binomial, Gamma, Gaussian Inverse or Tweedie. The identity link function was adopted in all cases. When differences were detected between the means, they were compared two by two with the test of the least significant differences (LSD). In a second analysis to evaluate the effect of the collection station (pre-pandemic and pandemic) generalized linear models with the same criteria previously adopted were also used, but in this

case the analyses were performed independently for each sector of the study. Additionally, the stations were also compared independently of the evaluation sector. The station factor was not included as a factor and in a possible interaction of the station with unit since the data present low sampling for the station. Despite this, the comparison within each unit could provide important information on the change of data as a function of the pandemic.

The correlations were tested with Pearson correlation, since the objective was to seek linear associations between the variables for use in linear regression models, regardless of the individual distribution of each variable. The significance of the correlation was tested with student's *t*-test.

For CFU/m³ prediction, the data were adjusted to simple and multiple linear regression models, based on the ordinary least squares method. The simple models were used for the selection of the variables of T, CO₂ and PM for the multiple models, and the variable with the highest individual coefficient of determination was adopted. To obtain the reduced model, we chose to maintain the most parsimonious model with only significant predictor variables, by the backward method.

For all analyses, the data were analyzed in the Software SPSS version 20.0 or in Environment R. The significance of 5% was adopted for all analyses, except when described.

3. Results

In the total qualitative bacterial assessment, 13 bacteria genera from the four critical units and nine from de external ambiance were isolated and identified. There is a striking dominance of Gram-Positive bacteria, mainly from the genus *Staphylococcus* sp, which are also noticed in the external ambiance control (Tables 1 and 2). Different pathogens of public health interest were identified. No bacteria specimens were isolated from the chromID VRE agar.

During the pre-pandemic state, Kidney-Transplanted, Clinical Oncology, and Burn and Plastic Surgery units have the lowest number of bacterial species isolated, six different species have been identified in each of these units. Approximately 67% of the airborne species found in the Kidney-Transplanted environment are from the genus *Staphylococcus* sp, that same proportion of *Staphylococcus* sp is found in Clinical Oncology. At Burn and Plastic Surgery unit, *Staphylococcus* sp, were encountered in lower proportion (\cong 33%); 40% of the total

bacteria identified in the Clinical Oncology unit are from the specie *S. xylosus*. At the Plastic Surgery unit, different species appear like *Chryseobacterium gleum*, *Micrococcus luteus*, and *Elizabethkingia meningosepti*. The external control for this site also varied from others due to the high fraction of the genus *Pseudomonas* discovered (Table 1).

In pre-pandemic, the specialized unit Infectious Disease presented the higher amount of bacterias isolated, 30 specimens from 17 different species, approximately 59% is referent to *Staphylococcus* sp, in greater quantity *S. haemolyticus* whose is also the most found in the external control for this unit. Species such *P. aeruginosa*, *Serratia marcescens* and *Acinetobacter baylyi* were also identified (Table 1). This unit also had the higher CFU/m³ of all units during this state (264.11±161.36); Kidney-Transplanted unit had the lowest CFU/m³ concentration (113.44±24.45) (Table 5).

In the pandemic state, a total of 30 bacteria were isolated and identified, the most common genera is *Bacillus* sp, (50%). In Infectious Disease unit, approximately 29% of the species identified is *Bacillus* sp, 40% of Kidney-Transplanted and Burn and Plastic Surgery, and 20% of the species encountered in Clinical Oncology were identified as *Bacillus* sp.

In comparison to the pre-pandemic, the Infectious Disease unit presented a notable decrease in the CFU/m³ concentration, in the number of species identified, with only seven identified in the pandemic state, as well as a reduction in the total number of bacteria isolated (8) (Table 2).

This downturn is also notable in the percentage of *Staphylococcus* sp ($\cong 29\%$), the species identified were *S. xylosus* and *S. haemolyticus* whereas in the control group from this unit, there is not a significant difference between pre-pandemic and pandemic states. This decrement pattern of bacteria from the genus *Staphylococcus* sp is outstanding throughout the remaining units at the pandemic stated, 40% of Clinical Oncology, and none from Kidney-Transplanted and Burn and Plastic Surgery corresponds to *Staphylococcus* sp (Table 2).

Unlike pre-pandemic, in the pandemic state The Kidney-Transplanted unit had the higher CFU/m³ concentration (100.89±25.95) and the Infectious Disease unit the lowest (51.22±8.89) (Table 5).

A correlation analysis was conducted between all the environmental parameters measured. For this assessment, the two states were analyzed as one group entirety (Table 3). Mean Particulate Matter (PM_m) propones a positive relation with occupancy, Minimum Particulate Matter (PM_{min}), and a weaker one with Maximum Particulate Matter concentration (PM_{max}). Momentary Temperature (T_{mon}) only showed a positive correlation when compared

to Minimum Temperature (Tmin) and Maximum Temperature (Tmax), likewise to Maximum Carbon Dioxide concentration (CO₂max) with Minimum Carbon Dioxide concentration (CO₂min) as to Mean Carbon Dioxide concentration (CO₂m); and mean CFU/m³ in comparison to RH and I/Eday. However, those correlations might be judged as weak and likely unimportant

Tmax is one of the two variables with a negative correlation, its relation with Tmin demonstrates a weak negative association with Tmin. The same result is recognized at variable CO₂m with occupancy, this same item set against CO₂min presented a forceful correlation.

A Multiple Linear Regression was used to test if the environmental variables significantly predicted CFU/m³ (Table 4). The overall regression was statistically significant with $R^2 = 0,17$, $p < 0,05$. CFU/m³ were predicted by the variables Tmin and RH.

A comparative analysis established in Table 5 allowed the creation of a parallel between indoor air quality (IAQ) in the pre-pandemic state and during the SARS-CoV-2 pandemic. The control group pre and pandemic only features statistically significantly different in the variable minimum, maximum, and mean CO₂ concentration. This pattern is recognizable through other units in the Table 5, although Clinical Oncology and Infectious Disease units' maximum CO₂ concentrations are not significantly different. All units had the concentration of Co₂ bellow the 1000 ppm proposed by Brazilian legislation (Anvisa, 2003)

Tmin, Tmax, and Tmon at Burns and PS unit and Tmin from Infectious Disease unit were higher at the pandemic stage. In contrast, Tmax from Infectious Disease and Clinical Oncology units have decayed during the pandemic. RH only exhibited significantly different results at the Kidney-Transplanted unit, whit a growth of over 40%, and at the Clinical Oncology unit whit a decrease of 17% at the RH mean, both during the pandemic state. The I/Eday were exclusively significant at the Infectious Disease unit. The variable occupancy was only significant when all units were analyzed in one group for each state.

4. Discussion

One of the main objectives of this research was to determine the type of bacteria present in the hospital environments and its risk to patients and healthcare staff. From the 13 genera identified in the hospital wards, 11 are of clinical significance, capable of causing pathogenic

and/or opportunistic infection risk for patients and healthcare workers, most of them Gram-positive bacteria.

The majority of these microorganisms are from the genus *Staphylococcus* sp, an opportunist group of Gram-positive bacteria that are part of the natural microbiota of skin and mucous membranes. The majority of *Staphylococcal* species encountered in the present study are coagulase-negative *Staphylococcus*. Though the virulence of this genus is considered relatively low, coagulase-negative *Staphylococcus* are widely described as Healthcare-Associated Infections (HAIs) causers in high-risk groups (Becker et al., 2014; Kozajda et al., 2019; Sivagnanasundaram et al., 2019). It is well described the participation of these *Staphylococcal* infections in bacteremia relating to catheters, surgical prostheses, pneumonia, urinary tract infection (UTI), septic arthritis, endophthalmitis, septic shock, brain abscess, peritonitis, endocarditis, and generally wound infections and is recognized to be significant contributors to morbidity and mortality. The hospital staff is established as a common carrier of them (Dadashi et al., 2015; Kozajda et al., 2019; Yousefzadeh et al., 2022).

Among these, the most prevailed is *S. haemolyticus*, found mainly in Infectious Diseases and in Clinical Oncology during the pre-pandemic state. These results are similar to publications by Yousefzadeh et al. (2022) who collected air samples with a one-stage sampler for a period of four months in the fall and winter of 2019, a total of 262 bacterial and fungal samples were collected from the air of the wards of educational Tohid Hospital, Sanandaj, Iran, 31.84% were *S. haemolyticus*.

Other important species of clinical significance found are *S. saprophyticus* (Kidney-Transplanted, pre-pandemic); *S. epidermidis* (Infectious Diseases, Kidney-Transplanted, and Burn and Plastic Surgery, pre-pandemic; Clinical Oncology, pandemic); *S. sciuri* (Infectious Diseases, pre-pandemic), and *S. hominis* (Burn and Plastic Surgery, pre-pandemic) (Becker et al., 2014). These commensal bacteria colonize different sites of the human body and can cause opportunist infections, mainly nosocomial infections transmitted by medical and/or nursing procedures (Becker et al., 2014).

S. intermedius (Infectious Diseases, pre-pandemic) is a coagulase-positive zoonotic pathogen, in literature, a few cases of infections in humans are found, and most of them are reported in association with animal exposure (Vandenesch et al., 1995; Kelesidis et al., 2010).

In comparison to the external control, except for *S. sciuri*, and *S. hominis* were encountered aerosolized in the outside air, however, *S. cohnii*, a coagulase-positive species of clinical interest, were only found in this ambiance.

Bacillus sp are Gram-positive bacteria with a high tolerance to severe environmental conditions and conventional disinfection procedures due to the ability to form spores. This microorganism can be part of the natural human microbiota, such as in the gastrointestinal tract, and offer a pathogenic risk only to immunocompromised patients (Sivagnanasundaram et al., 2019; Yousefzadeh et al., 2022).

In this study, *Bacillus* sp with clinical significance were found in almost every hospital sampling sites including in the control groups. In pre-pandemic, *B. megaterium* (Infectious Diseases, Clinical Oncology) and *B. pumilus* (Infectious Diseases, Burn and Plastic Surgery) appear in higher concentrations. However, during the pandemic, *Bacillus cereus* emerged as a prevalent human pathogenic contaminant in every unit, except for Infectious Disease, in that area was found only *B. pumilus* and *B. megaterium*.

B. megaterium is generally considered to be non-pathogenic and of low virulence, but there are cases in the literature that reported eye, skin, brain, and pleuritis infections caused by this bacteria (Duncan & Smith, 2011; Guo et al., 2015; Crisafulli et al., 2018). *B. pumilus* is usually found in the soil as a commensal and are more typically isolated in cultures as contaminants, but rarely implicated as a pathogen (Shivamurthy et al., 2016). *B. cereus* is associated with food poisoning, nosocomial bacteremia, pneumonia, wound infections, and central nervous system infections (Sivagnanasundaram et al., 2019).

M. luteus were isolated in Burn and Plastic Surgery unit during pre-pandemic state. It is considered an emerging nosocomial pathogen capable of causing bacteremia, septic shock, septic arthritis, endocarditis, meningitis, intracranial suppuration, and cavitating pneumonia ((Sivagnanasundaram et al., 2019; Yousefzadeh et al., 2022). Burn injuries possess a high risk of complications involving Gram-positive infections as result of the trauma caused to the skin barrier that protects the body from external conditions, along with their relative immunocompromised state (Schultz et al., 2013; Torres et al., 2020; Kelly et al., 2022). *M. luteus* offer a great contamination risk to burn patients due to its presence in the microflora surrounding the wound bed (Church et al., 2006; Kelly et al., 2022).

Aerococcus viridans is the last Gram-positive bacteria with clinical significance found in the Kidney-Transplanted unit during pre-pandemic state. *A. viridans* is an airborne organism common in hospitals and health care facilities, even though these bacteria rarely cause human infections there are cases reported to cause infective endocarditis, spondylodiscitis, and UTIs (Rasmussen, 2015; Ezechukwu et al., 2019).

Gram-negative bacteria are becoming increasingly resistant to known antibiotics due to their growing use globally. Patients with Chronic diseases, who are repeatedly hospitalized, are at greater risk of contracting multiple Gram-negative bacterial throughout their hospitalization history (Agarwal et al., 2018). In this study, Gram-negative microorganisms with clinical significance appeared in lower concentrations when compared to Gram-positive bacteria. *P. aeruginosa* was isolated from the Infectious Disease unit, pre-pandemic, this is a Gram-Negative opportunistic pathogenic bacteria capable of cause a vast range of infections. *P. aeruginosa* is responsible for about 10% of all HAIs worldwide and is associated with nosocomial outbreaks in adult, pediatric, and neonatal intensive care units (ICUs) as a result of the spread of multi-drug resistant or extensively drug-resistant and highly virulent strains (Azan and Khan, 2018; Rodrigues et al., 2020; Petitjean et al., 2021). *P. aeruginosa* have been related to hospital-associated pneumonia, gastrointestinal infections, dermatitis, UTIs, skin infections, soft tissue infections and several other infections especially in patients with severe burns, and immunocompromised patients, such as those with cancer or AIDS.

From the same genus, *P. fulva* was encountered in pre-pandemic at the Clinical Oncology unit, and during pandemic at the Infectious Disease unit. This species appears in various environments and is a rare opportunistic pathogen for humans with only a few cases described in the literature, normally associated to trauma and invasive procedures (Cobo et al., 2016; Uddin et al., 2018; Stark et al., 2022). Five other species of *Pseudomonas* sp were isolated from the external control in the two parts of this work, *P. oryzihabitans* (formerly known as *Flavimonas oryzihabitans*) (Lin et al., 1997; Nei et al., 2015), *P. mosselii* (Leneveu-Jenvrin et al., 2013), *P. monteilii* (Remold et al., 2011; Aditi et al., 2017; Toledo et al., 2022), *P. putida* (Bogaerts et al., 2008; Remold et al., 2011; Kivisaar, 2020), *P. stutzeri* (Noble & Overman, 1994; Lalucat et al., 2006; Alwazzeh et al., 2020), all classified as rare opportunistic pathogens for humans.

Serratia marcescens (Infectious Disease, pre-pandemic; Burn and Plastic Surgery, pandemic) is an opportunistic and nosocomial pathogen. It is related to pneumonia, urinary tract infections, bacteremia, meningitis, and endocarditis, particularly in immunocompromised patients as a result of the cytotoxic effect on human epithelial cells leading to vacuolization and cell lysis. *S. marcescens* is commonly resistant to penicillins and first- and second-generation cephalosporins, although new antimicrobial strains have appeared widely (Fazio et al., 2021; Ono et al., 2022).

Acinetobacter baumannii (Burn and Plastic Surgery, pandemic) is one of the most challenging pathogens in healthcare environments due to its predisposition for ample antimicrobial resistance with the capacity to develop mechanisms to resist desiccation and disinfection regimes of hospital ambiance. *A. baumannii* infections occur mainly among patients in long-term hospitalization, particularly those who are immunocompromised or receive treatment in intensive care units. Furthermore, this bacteria can lead to various diseases such as pneumonia, osteomyelitis, peritonitis, endocarditis, septicemia, meningitis, and wound, skin, soft tissue, urinary tract, ear, and eye infections (Chusri et al., 2019; Nguyen & Joshi, 2021). As discussed previously, *A. baumannii* infections are alarming to patients with major trauma such as burns injuries, this bacteria was isolated only from Burn and Plastic Surgery unit in the pandemic state, which suggests a great risk to the patients admitted to this unit. *Acinetobacter baylyi* (Infectious Disease, pre-pandemic) is rarely associated with human infections (Chen et al., 2008).

Chryseobacterium gleum (Burn and Plastic Surgery, pre-pandemic) are Gram-negative bacilli that aren't a part of human microflora and are found frequently in soil and water. Its opportunistic risk is mainly for patients with long-term hospitalizations, principally in Intensive Care Units, which are related to HAIs, including septicemia, pneumonia, UTIs, wound infections, peritonitis, and meningitis (Amisha et al., 2021).

Similarly, *Elizabethkingia meningoseptica* (Burn and Plastic Surgery, pre-pandemic), bacteria from this genus have already been associated with the hospital environment and opportunistic infections such as meningitis, bacteremia, pneumonia, skin and soft-tissue infection, catheter-associated infection, and UTIs in neonates, infants, and immunocompromised patients (Bloch et al., 1997; Lin et al., 2018, Lin et al., 2019;)

Klebsiella variicola (Clinical Oncology, pandemic state) is a Gram-negative bacilli identified in several opportunistic human infections, mainly associated with HAIs, the pathogen has been described as responsible for causing respiratory tract infections, bloodstream infections, and UTIs. *K. variicola* is responsible for causing fewer nosocomial infections when compared to *K. pneumoniae*, however, they are capable of producing infections with the same risk and severity increasing morbidity and mortality (Rodríguez-Medina et al., 2019).

Pantoea sp is a bacillus encountered in diverse environments, including hospital buildings. It is not common to have immunocompetent patients with infection cases from this genus, but there are reports of opportunistic infections involving some species. In the pandemic state, two species of clinical interest were isolated, *P. agglomerans* (Infectious Disease; Burn

and Plastic Surgery, pandemic) and *P. dispersa* (Kidney-transplanted, pandemic), these microorganisms are related to a few nosocomial infections affecting immunocompromised individuals of different ages, most of them hospitalized in intensive care units multiple ages (Asai et al., 2019; Kaur et al., 2020; Mani & Nair, 2021).

It's been worldwide researched and described the participation of bacterial bioaerosols in HAIs despite that, there is no Brazilian legislation that imposes and regulates limits on airborne bacterial concentrations in indoor air (Brasil, 2003). Among the four hospital units and external control, the concentration of bacteria varied from 51.22 ± 8.89 to 264.11 ± 161.36 CFU/m³. The Infectious Disease unit had the highest concentration of CFU/m³ in the pre-pandemic state, and the Kidney-Transplanted unit reached the highest concentration in the pandemic state.

In Table 5, is noticeable that all units presented a decrease in the CFU/m³ concentration when comparing the pre-pandemic state and pandemic state. A possible explanation for this behavior is the adoption of new cleaning protocols due to the pandemic by the new coronavirus (*SARS-CoV-2*). The Hospital Foundation of the State of Minas Gerais (Fhemig) in the Guidelines Protocol of Assistance to fight *SARS-CoV-2* recommends an increase in the disinfection frequency of hospital units to 3 times a day, with alcohol 70% and another disinfectant standardized by the Health Service, preferably Sodium hypochlorite at 1 mg/liter (FHEMIG, 2021). The *SARS-CoV-2* Contingency Plan, written by HC-UFU (2021) chose to use disinfectants from the brands Surfânios® and Vulcan Hospitalar® as standard surface disinfectants in all inpatient units, including the ones reserved for *SARS-CoV-2*, but the document does not describe dilution concentration of these products.

The required concentration of free residual chlorine to inactivate the human coronavirus is 0.5 mg/liter (Wang et al., 2020), but there are descriptions of protocols that use concentrations greater than 6.5 mg/liter, the excess of this chemical may have contributed for the decline of bacterial CFU/m³, and for the reduction of microorganisms isolated for bacterial identification through MALDI-TOF MS (Lu & Guo, 2021). However, disinfectants facilitate the acquisition and spread of antimicrobial resistance (AMR) through mutation or horizontal gene transfer, providing a new risk environment and public health (O'Neil, 2016; Wang et al., 2020).

Another explanation may be the implementation of mandatory use of face masks through both the Guideline by FHEMG (2021) and the Contingency Plan by HC-UFU (2021). An article published by Jeong et al. (2021) attests to the filtration ability of microorganisms in different types of face masks, including Respirators Mask Filters (FFRs) of types N95 (FRR1),

KF94 produced by LG (FRR2), KF94 produced by Jeil Pharmaceutical Co. and Surgical Masks (SM) produced by Yuhan-Kimberly (SM1), and by 3M Nexcare (SM2), the authors also analyzed the microbial survival rate at each layer of FFRs and SMs. The FFR-type masks obtained a filtration >94%, efficiency in terms of bacterial aerosolization, with lower efficiency were the SMs-type face masks that may not be adequate to prevent formation and exposure of bacterial bioaerosols regardless, the microbial survival rate for all tested masks was >13%, regardless of filtration performance. Assuming the influence of the protocols implemented for facing *SARS-CoV-2*, a relationship can be established with the decrease in CFU/m³ bacteria isolated in each analyzed unit, which may explain the large reduction of CFU/m³, widely in the Infectious Disease unit that went from the highest CFU/m³ (264.11±161.36) to the lowest (51.22±8.89) during the SARS-CoV-2.

It is established that weather conditions can have an influence on bioaerosols concentrations, meteorological variations can initiate the dispersion of biological components that composes bioaerosols and cause discomfort to the patients and hospital staff. Brazilian legislation recommends that the temperature in indoor spaces of the public domain remain between 23° to 26° C with a maximum operating range of 26.7° to 27° C. For RH the percentage varies from 40% to 65%. The specific range selection for temperature and relative humidity depends on the purpose and facility location (Brazil, 2003). Table 4 evidence that CFU/m³ is predicted by the variables T_{min} and RH, in addition to this, the data presented in Table 5 showed that almost no unit stayed between the standards stipulated by the country's legislation for these variables. In relation to RH, Table 3 also demonstrates a correlation between this variable with CFU/m³ and IE/day.

Infectious Disease exemplified the lowest T_{min} (21.17±1.67) between the units analyzed in the pre-pandemic state (Table 5), being statistically significantly different when compared to the pandemic state (26.83±0.87), that condition may explain why the unit had the highest level of contamination (264.11±161.36) and greater diversity of bacterial species identified (17) in the pre-pandemic and its decrease in the pandemic. The variable I/E_{day} only presented significantly different in the Infectious Disease unit, which also presented an RH higher than the recommended by the law in both states, even though the states did not significantly differ between themselves.

As known, particulate matter (PM) varies due to the influence of the number of patients, staff, and other persons in the hospital wards, along with factors like ventilation systems and approximation to external fonts of emission (Fiordelisi et al., 2017; Losacco & Perillo, 2018;

Kim et al., 2020). A positive correlation between occupancy and PM₁₀ (Table 3) was encountered in the present study even though all units presented a PM concentration under 80 mg/m³, the maximum proposed by ANVISA (2003). Additionally, there is no explicit relationship between this and the other variables analyzed, and also what is its impact on CFU/m³, a similar result was proposed by Yousefzadeh et al (2022).

Even though the analysis realized in this assessment is complete, a deeper evaluation is recommended to study influential factors such as the effect of all seasons, working shifts, source of air contamination, and natural air flow should be realized for a more complete view of the bioaerosol composition and possible adoption of IAQ control for each specialized unit studied and other sectors of the hospital individually.

5. Conclusion

Of the 13 genera encountered in the four hospital units analyzed, 11 are classified as opportunistic and/or pathogenic bacteria with a dominance of Gram-positive bacteria, mainly *Staphylococcus* sp but also *Bacillus* sp, *M. luteus*, *A. viridians*. Gram-negative bacteria, such as *Pseudomonas* sp, *A. baumannii* and *K. variicola*, were encountered in lower concentration.

In the pre-pandemic state, the majority of bacteria identified were from the Infectious Disease unit, which also had the higher concentration of colony-forming units (CFU/m³), and the Kidney-Transplanted unit had the lowest CFU/m³. In the pandemic state, the CFU/m³ and the number of bacteria identified in the qualitative evaluation from Infectious Disease presented a considerable decrease in the pandemic state even though this pattern occurred in lower intensity in all units; Kidney-Transplanted had the higher concentration of CFU/m³.

The correlation analysis showed a positive relationship between CFU/m³ to Relative Humidity (RH) and Indoor and External air daily relationship (IE/day), the multiple linear regression showed that Minimum Temperature (T_{min}) predicts CFU/m³ and RH. The infectious Disease unit had the lowest T_{min} in pre-pandemic and was the only one with an IE/day relationship statistically significantly. Occupancy had a positive relationship with Mean Particulate Matter (PM₁₀). The variables of temperature and relative humidity were commonly encountered above the recommended.

In view of the above, this study demonstrates the need to monitor hospital Indoor Air Quality (IQA) and bacterial bioaerosol, allowing greater safety due to possible air contamination in people who use and work in these environments.

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Conflict of interest The authors declare no conflict of interest.

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Table 1: Pre-pandemic bacterial species identified from Clinical Hospital of the Federal University of Uberlândia specialized critical hospitalization units and external control between November 2019 and February 2020.

UNITS	BACTERIAL SPECIES ISOLATED FROM CRITICAL UNITS (*)	BACTERIAL SPECIES ISOLATED FROM EXTERNAL CONTROL**
INFECTIOUS DISEASE	<i>Staphylococcus haemolyticus</i> (6)	<i>Staphylococcus haemolyticus</i> (4)
	<i>Staphylococcus intermedius</i> (3)	<i>Staphylococcus intermedius</i> (2)
	<i>Staphylococcus epidermidis</i> (1)	<i>Staphylococcus epidermidis</i> (1)
	<i>Staphylococcus hyicus</i> (1)	<i>Pseudomonas oryzae</i> (1)
	<i>Staphylococcus xylosus</i> (1)	<i>Acinetobacter ursingii</i> (1)
	<i>Staphylococcus equorum</i> (1)	<i>Staphylococcus arlettae</i> (1)
	<i>Staphylococcus sciuri</i> (1)	<i>Staphylococcus piscifermentans</i> (2)
	<i>Staphylococcus caprae</i> (1)	<i>Staphylococcus saprophyticus</i> (4)
	<i>Pseudomonas aeruginosa</i> (3)	<i>Exiguobacterium aurantiacum</i> (1)
	<i>Serratia marcescens</i> (1)	<i>Pseudomonas fulva</i> (1)
	<i>Bacillus pumilus</i> (3)	
	<i>Staphylococcus arlettae</i> (2)	
	<i>Staphylococcus gallinarum</i> (1)	
	<i>Bacillus megaterium</i> (2)	
	<i>Bacillus safensis</i> (1)	
<i>Bacillus altitudinis</i> (1)		
<i>Acinetobacter baylyi</i> (1)		
KIDNEY-TRANSPLANTED	<i>Staphylococcus equorum</i> (1)	<i>Staphylococcus nepalensis</i> (1)
	<i>Oceanobacillus caeni</i> (1)	<i>Staphylococcus xylosus</i> (3)
	<i>Aerococcus viridans</i> (1)	<i>Pseudomonas fulva</i> (1)
	<i>Staphylococcus saprophyticus</i> (1)	<i>Staphylococcus cohnii</i> (1)
	<i>Staphylococcus arlettae</i> (1)	
<i>Staphylococcus epidermidis</i> (2)		
CLINICAL ONCOLOGY	<i>Staphylococcus xylosus</i> (4)	<i>Staphylococcus xylosus</i> (5)
	<i>Staphylococcus haemolyticus</i> (1)	<i>Pseudomonas mosselii</i> (1)
	<i>Staphylococcus arlettae</i> (1)	<i>Kosakonia cowanii</i> (2)
	<i>Bacillus megaterium</i> (2)	<i>Pantoea ananatis</i> (2)
	<i>Staphylococcus napelensis</i> (1)	<i>Pseudomonas monteilii</i> (1)
	<i>Pseudomonas fulva</i> (1)	<i>Escherichia vulneris</i> (2)
	<i>Bacillus megaterium</i> (1)	
	<i>Acinetobacter radioresistens</i> (1)	
BURN AND PLASTIC SURGERY	<i>Staphylococcus hominis</i> (1)	<i>Staphylococcus cohnii</i> (1)
	<i>Chryseobacterium gleum</i> (1)	<i>Bacillus megaterium</i> (2)
	<i>Staphylococcus epidermidis</i> (1)	<i>Staphylococcus gallinarum</i> (5)
	<i>Micrococcus luteus</i> (1)	<i>Pseudomonas putida</i> (1)
	<i>Bacillus pumilus</i> (1)	<i>Pseudomonas stutzeri</i> (1)
	<i>Elizabethkingia meningosepti</i> (1)	<i>Pseudomonas oryzae</i> (2)

(*): Number of microorganisms from each species isolated and identified. **External control realized at the Hospital building concierge.

Table 2: Pandemic bacterial species identified from Clinical Hospital of the Federal University of Uberlândia specialized critical hospitalization units and external control between November 2020 and February 2021.

UNITS	BACTERIAL SPECIES ISOLATED FROM CRITICAL UNITS (*)	BACTERIAL SPECIES ISOLATED FROM EXTERNAL CONTROL**
INFECTIOUS DISEASE	<i>Bacillus megaterium</i> (1)	<i>Bacillus megaterium</i> (6)
	<i>Staphylococcus xylosus</i> (1)	<i>Pseudomonas oryzihabitans</i> (3)
	<i>Pseudomonas fulva</i> (1)	<i>Staphylococcus xylosus</i> (1)
	<i>Pantoea agglomerans</i> (1)	<i>Pantoea gaviniae</i> (1)
	<i>Staphylococcus haemolyticus</i> (1)	<i>Escherichia vulneris</i> (1)
	<i>Bacillus pumilus</i> (2)	<i>Staphylococcus lentus</i> (1)
	<i>Paenibacillus lactis</i> (1)	<i>Bacillus pumilus</i> (1)
		<i>Bacillus cereus</i> (1)
		<i>Paenibacillus lactis</i> (1)
KIDNEY-TRANSPLANTED	<i>Bacillus cereus</i> (3)	<i>Pantoea dispersa</i> (2)
	<i>Pseudomonas monteilii</i> (1)	<i>Bacillus megaterium</i> (1)
	<i>Pantoea dispersa</i> (1)	<i>Staphylococcus xylosus</i> (1)
	<i>Bacillus licheniformis</i> (1)	<i>Pseudomonas oryzihabitans</i> (1)
	<i>Pseudomonas stutzeri</i> (1)	<i>Staphylococcus haemolyticus</i> (1)
CLINICAL ONCOLOGY	<i>Staphylococcus epidermidis</i> (1)	<i>Bacillus cereus</i> (5)
	<i>Staphylococcus xylosus</i> (1)	<i>Pantoea agglomerans</i> (1)
	<i>Bacillus cereus</i> (2)	<i>Staphylococcus xylosus</i> (1)
	<i>Pseudomonas stutzeri</i> (1)	<i>Bacillus licheniformis</i> (1)
	<i>Klebsiella variicola</i> (1)	<i>Pseudomonas putida</i> (1)
BURN AND PLASTIC SURGERY	<i>Bacillus cereus</i> (5)	<i>Bacillus cereus</i> (3)
	<i>Bacillus subtilis</i> (1)	<i>Escherichia vulneris</i> (1)
	<i>Pantoea agglomerans</i> (1)	<i>Pantoea séptica</i> (1)
	<i>Acinetobacter baumannii</i> (1)	
	<i>Serratia marcescens</i> (1)	

(*): Number of microorganisms from each species isolated and identified. **External control realized at the Hospital building concierge.

Table 3. Pearson correlation (rho) from the environmental variables analyzed.

Trait	Statistic	Occupancy	PMmin	PMm	PMmax	Tmin	Tmax	Tmom	RH	CFUm	CO ² min	CO ² m	CO ² max
PMm	<i>rho</i>	0.606	0.566										
	<i>p</i>	0.002	<0.001										
PMmax	<i>rho</i>	0.322	-0.011	0.304									
	<i>p</i>	0.124	0.939	0.036									
Tmin	<i>rho</i>	0.102	-0.074	0.051	0.001								
	<i>p</i>	0.636	0.618	0.731	0.993								
Tmax	<i>rho</i>	0.130	0.089	0.141	0.075	-0.304							
	<i>p</i>	0.545	0.547	0.338	0.612	0.035							
Tmom	<i>rho</i>	0.256	-0.068	0.099	0.154	0.403	0.46						
	<i>p</i>	0.227	0.646	0.502	0.295	0.005	0.001						
RH	<i>rho</i>	0.105	0.111	0.152	-0.039	0.081	-0.197	-0.068					
	<i>p</i>	0.626	0.454	0.302	0.792	0.586	0.179	0.645					
CFU/m ³	<i>rho</i>	0.073	-0.056	-0.009	0.055	-0.274	0.052	-0.268	0.340				
	<i>p</i>	0.735	0.705	0.951	0.710	0.060	0.725	0.066	0.018				
CO ₂ min	<i>rho</i>	-0.438	-0.062	-0.213	-0.042	0.134	-0.273	-0.023	0.15	-0.11			
	<i>p</i>	0.033	0.678	0.146	0.775	0.365	0.060	0.877	0.31	0.458			
CO ₂ m	<i>rho</i>	-0.435	-0.071	-0.235	-0.045	0.035	-0.234	-0.069	0.087	-0.113	0.953		
	<i>p</i>	0.033	0.630	0.108	0.762	0.813	0.109	0.641	0.557	0.445	<0.001		
CO ₂ max	<i>rho</i>	-0.156	-0.061	-0.151	-0.073	-0.113	0.001	-0.057	0.129	0.148	0.401	0.566	
	<i>p</i>	0.468	0.679	0.307	0.621	0.443	0.992	0.698	0.381	0.316	0.005	<0.001	
I/Eday	<i>rho</i>	-0.044		0.005	0.221	-0.276	-0.275	-0.267	0.443	0.585	-0.181	-0.155	0.068
	<i>p</i>	0.839		0.98	0.299	0.191	0.194	0.208	0.030	0.003	0.398	0.469	0.752

p: probability based on Student's *t*'test. Pearson correlation of Occupancy; Minimum Particulate Matter (PMmin), Mean Particulate Matter (PMm), and Maximum Particulate Matter concentration (PMmax); Minimum Temperature (Tmin), Maximum Temperature (Tmax), and Momentary Temperature (Tmom); Relative Humidity; Mean Colony-Forming Units per cubic meter (CFU/m³); Minimum Carbon Dioxide concentration (Co²min), Mean Carbon Dioxide concentration (Co²m), and Maximum Carbon Dioxide concentration (Co²max), Indoor and external air daily relationship (IEday), the two phases were analyzed the entirety. *p*: <0,05.

Table 4. Reduced Model of Multiple Linear Regression applied to CFU/m³

Preditor	Reduced Model					
	<i>Bi</i>	<i>EP</i>	<i>p</i>	<i>LI IC95%</i>	<i>LS IC95%</i>	<i>R2</i>
Constant	196.55	98.12	0.051	-1.073	394.173	0.171
Mimumum Temperature	-8.77	3.85	0.028	-16.527	-1.004	
Relative Humidity	1.62	0.59	0.009	0.427	2.818	

Bi: i-th parameter estimate; *SE*: standard error; *p*: Student's t tested-based probability, *LI*: lower limit; *LS*: upper limit; *CI95%*: confidence interval at 95%. $P < 0,05$.

Table 5. Variables comparison from the four specialized critical units analyzed during pre-pandemic state and the pandemic state mean (\pm Standard error).

Trait	Burn and Plastic Surgery		Control		Infectious Disease		Kidney-Transplanted		Clinical Oncology		All units	
	*Pre	**Pandemic	*Pre	**Pandemic	*Pre	**Pandemic	*Pre	**Pandemic	*Pre	**Pandemic	*Pre	**Pandemic
Occupancy	2 \pm 0	1 \pm 0			2.67 \pm 0.33	3 \pm 0	3.33 \pm 0.88 a	1.33 \pm 0.33 b	3.67 \pm 1.2	2.67 \pm 0.67	2.92 \pm 0.38 a	2 \pm 0.3 b
PMmin	0 \pm 0	0 \pm 0	0.50 \pm 0.42	0.5 \pm 0.42	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0.25 \pm 0.21	0.25 \pm 0.21
PMm	1 \pm 0	3.67 \pm 2.67	5.17 \pm 3.13	5.67 \pm 1.47	3 \pm 1.53	1 \pm 0	1 \pm 1	0.67 \pm 0.33	16.67 \pm 11.89	2.67 \pm 1.67	5.29 \pm 2.2	3.83 \pm 0.9
PMmax	8.33 \pm 1.2	51 \pm 41	19.17 \pm 4.81	31.75 \pm 8.65	22.67 \pm 10.4	9 \pm 2.08	34 \pm 19.5	6.33 \pm 0.88	52.67 \pm 26.28	30 \pm 22.01	24.29 \pm 5.02	27.92 \pm 7.02
Tmin	23.9 \pm 0.71 b	27.53 \pm 0.3 a	22.85 \pm 0.77	23.23 \pm 0.92	21.17 \pm 1.67 b	26.83 \pm 0.87 a	21.9 \pm 1.59	22.57 \pm 1.35	25.2 \pm 1.62	24.57 \pm 1.4	22.95 \pm 0.54	24.3 \pm 0.62
Tmax	26.67 \pm 0.81 b	29.17 \pm 0.12 a	29.29 \pm 0.88	28.43 \pm 0.6	31.43 \pm 1.17 a	28.53 \pm 1.04 b	28.67 \pm 2.19	25.77 \pm 0.64	30.63 \pm 0.83 a	28.13 \pm 0.46 b	29.32 \pm 0.59	28.16 \pm 0.38
Tmom	25.07 \pm 0.63 b	28 \pm 0.29 a	26.48 \pm 0.83	26.78 \pm 0.43	26.87 \pm 1.1	27.5 \pm 0.97	24.7 \pm 0.36	23.53 \pm 1.2	28.2 \pm 0.9	26.13 \pm 1.05	26.35 \pm 0.48	26.53 \pm 0.39
RH	65.33 \pm 17.53	72.33 \pm 6.49	72.33 \pm 5.61	77.33 \pm 3.41	82.33 \pm 9.26	68.33 \pm 3.76	44.33 \pm 18.21 b	82.67 \pm 10.9 a	87.33 \pm 2.67 a	70.33 \pm 9.17 b	71.08 \pm 4.66	75.38 \pm 2.54
CFU/m ³	133.78 \pm 3.,54	93.67 \pm 23.24	102.46 \pm 16.86	90.03 \pm 7.71	264.11 \pm 161.36	51.22 \pm 8.89	113.44 \pm 24.45	100.89 \pm 25.95	114 \pm 21.66	97.33 \pm 10.74	129.4 \pm 22.15	87.9 \pm 6.2
CO ₂ min	336.89 \pm 3.23 b	401.56 \pm 3.92 a	351.79 \pm 3.09 b	405.75 \pm 4.55 a	368.22 \pm 8.65 b	414.5 \pm 1.39 a	331.33 \pm 6.19 b	460.39 \pm 10.8 a	348.33 \pm 8.85 b	408.94 \pm 11.77 a	348.99 \pm 3.03 b	413,55 \pm 4,7 a
CO ₂ m	359.78 \pm 3.89 b	412.28 \pm 7.54 a	384.63 \pm 6.02 b	417.21 \pm 5.36 a	396.89 \pm 2.26 b	426.33 \pm 3.06 a	355.06 \pm 4.41 b	479.72 \pm 12.21 a	372.06 \pm 7.15 b	419.83 \pm 10.34 a	377.78 \pm 4.17 b	425,88 \pm 5,38 a
CO ₂ max	384.78 \pm 4.02 b	441.17 \pm 11.75 a	459.86 \pm 5.25 a	432.97 \pm 5.24 b	483.39 \pm 12.89	461.39 \pm 18.69	433.17 \pm 9.91 b	521.06 \pm 4.14 a	456.56 \pm 13.95	438.78 \pm 9.48	449.67 \pm 6.69	449,28 \pm 6,97
IEday	1.64 \pm 0.58	1.23 \pm 0.38			1.43 \pm 0.37 a	0.5 \pm 0.06 b	1.32 \pm 0.35	1.4 \pm 0.35	1.59 \pm 0.31	1.04 \pm 0.26	1.49 \pm 0.18	1,04 \pm 0,16

¹ Values followed by different letters in each unit differs based on Generalized Linear Model ($\alpha = 0.05$). *Pre-pandemic state, between November 2019 and February 2020. **Pandemic state, between November 2020 and February 2021. Mean (\pm Standard error) from Occupancy, Minimum Particulate Matter (PMmin), Mean Particulate Matter (PMm), and Maximum Particulate Matter concentration (PMmax); Minimum Temperature (Tmin), Maximum Temperature (Tmax), and Momentary Temperature (Tmom); Relative Humidity; Mean Colony-Forming Units per cubic meter (CFU/m³); Minimum Carbon Dioxide concentration (Co²min), Mean Carbon Dioxide concentration (Co²m), and Maximum Carbon Dioxide concentration (Co²max); Indoor and external air daily relationship (IEday).

