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MICROBIOLOGY

Detection of Waterborne and Airborne Microorganisms in a Rodent Facility

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Abstract: This study aimed to evaluate the air and water contamination level and to identify the microbes isolated from a rodent facility located at the Federal University of Uberlândia, Minas Gerais, Brazil. Colony forming units (CFU) per milliliter was used for monitoring water quantitatively; CFU per cubic meter was used for air monitoring. The isolated colonies were identified for qualitative monitoring. Due to absence of specific parameters for these facilities, the results were analyzed according to Brazilian and international standards, depending on which best suited each sample. The mean total number of microorganisms in water ranged from 0.015 ± 0.02 to 0.999 ± 0.91 CFU/mL. The number of microorganisms in air ranged from 9.1 ± 4.6 to 351.56 ± 158.2 CFU/m³. Forty-one microorganisms identified in the samples obtained from the rodent facility were potentially pathogenic or opportunistic for animals and humans (e.g., *Corynebacterium* spp.). We concluded that the water and air samples were contaminated with potentially pathogenic or opportunistic microorganisms that can harm rodents and humans. On the basis of our observations, specific sanitary standards suitable for these facilities should be developed for controlling microbial contamination, which will prevent zoonosis and ensure the reliability of scientific results obtained from animal experiments.

Key words: waterborne, airborne, rodent facility, fungi, bacteria, microbial.

INTRODUCTION

Biological contamination in facilities used for housing animals intended for scientific research prevents good laboratory quality from being achieved and maintained (Majerowicz 2019). Constantly surveilling the presence of microbes in these environments is required given that microorganisms can cause serious diseases in humans and animals and may negatively affect the quality of research conducted with these animals (Felasa 2014, Majerowicz 2019). Therefore, Brazilian animal facilities are designed to comply with regulations, legislation, and standards created to meet the needs of animal and scientific research (Politi et al. 2008, Brasil 2008).

Quality control programs aimed at maintaining animal health have been designed (Felasa 2014). However, the animal facility environment must also comply with quality control standards because water and air are sources of microbial contamination (Cincinelli & Martellini 2017, Dawson & Sartory 2000, Kauffmann-Lacroix et al. 2016, Rao et al. 1996, Strickland & Shi 2021, Zhou et al. 2020). Although animal facilities are known to be sources of contamination, the Brazilian legislation does not define specific standards for their environmental quality control.

Indoor air contamination occurs via bioaerosols, which, in most cases, are generated in the external environment and carried inside

by people, ventilation systems, or doors and windows (Mirskaya & Agranovski 2018, Zhou et al. 2020). Continuous exposure of employees and animals to possible bacterial endotoxins and exotoxins compromises the immune system, causing respiratory and gastrointestinal diseases such as pulmonary emphysema and intestinal inflammation (Lai et al. 2016).

Feces-contaminated water can cause serious diseases, including cholera and typhoid fever, in humans and animals. Hence, the absence of fecal coliforms is used as a marker to verify water safety (Boelee et al. 2019, Leclerc et al. 2001, Nowicki et al. 2021). However, the presence of fungi in water cannot be ignored because they may be pathogenic, especially in immunosuppressed organisms. The ability to form biofilms on surfaces and thrive even in nutrient-poor places primarily account for bacterial and fungal contamination in water (Kauffmann-Lacroix et al. 2016, Edstrom & Curran 2003). Fungi belonging to the genus *Aspergillus* spp. are mainly responsible for disease outbreaks, and their dissemination is related to transmission not only by contaminated water, but also by air, through bioaerosols (Kauffmann-Lacroix et al. 2016).

Several studies have identified pathogenic and other fungi and bacteria in animal facilities for rodents (Carriquiriborde et al. 2020, Na et al. 2010, Kunstyr et al. 1997), and quality control recommendations for ensuring rodent health have been implemented. Nevertheless, these parameters do not cover the entire environment of animal facilities (Felasa 2014, Na et al. 2010, Mailhiot et al. 2020). Information regarding monitoring and maintenance of air and water microbial quality is currently available (Dawson & Sartory 2000, Kim et al. 2018, Leclerc et al. 2001, Edstrom & Curran 2003, Westall et al. 2015), which is essential for formulating ways to detect and to control infections caused by pathogenic and opportunistic microorganisms in animal facilities (Schlapp et al. 2018, Cincinelli & Martellini 2017, Mansfield et al. 2010, Kunstyr et al. 1997, Ooms et al. 2008, Westall et al. 2015).

To minimize occupational risks, ensure scientific quality, and identify possible contamination points, in this study we aimed to evaluate the presence of bacterial and fungal contaminants in the air and water of a rodent facility used for scientific research.

MATERIALS AND METHODS

Rodent facility characterization

The rodent facility analyzed in the present study has a total area of 733 m² and is divided into several rooms. Figure 1 shows the floor plan of the evaluated rooms and their surroundings. Air circulation in the rooms occurs through common air conditioning. Employees circulating in the breeding area wear disposable and sterile clothing, gloves, and caps. The rodents are kept in individual ventilation racks equipped with HEPA filter (Tecniplast SpA, Buguggiate, Varese, Italy). The rodents are maintained at 40–60% humidity, 20±1°C, and light and dark (12/12) cycles, and the filters are changed annually. The cages and the wood shavings are sterilized. The rodents receive feed and filtered and sterilized water *ad libitum*. The cages are cleaned and changed weekly.

The facility is cleaned and disinfected with Virkon's solution (Antec International, Sudbury, Suffolk, United Kingdom) along six months and with hypochlorite (Uzzi Quimica Ltda, Uberlândia, Minas Gerais, Brazil) along the following six months to prevent microorganisms from creating resistance to disinfecting agents.

Collection and sampling locations

All the samples were obtained from the Central Animal Facility of the Animal Facility Network

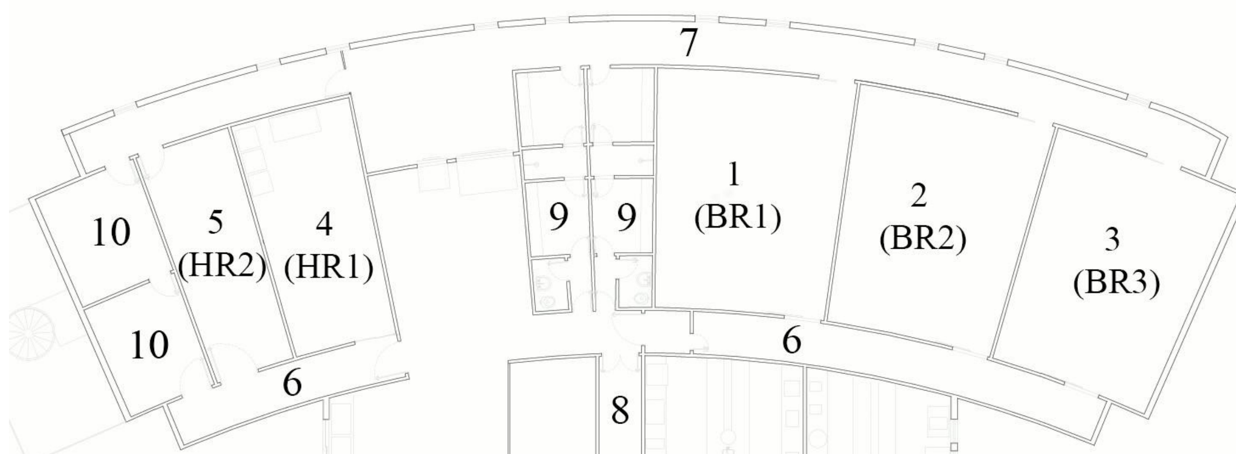

Figure 1. Rodent facility floor plan. 1. breeding room 1; 2. breeding room 2; 3. breeding room 3; 4. housing room 1; 5. housing room 2; 6. clean corridors; 7. dirty corridors; 8. area entrance; 9. restrooms and staff locker room; 10. storage room.

of the Federal University of Uberlândia, Minas Gerais, Brazil. Air samples were collected from three 6 x 6 m² breeding rooms, which were designated breeding room 1 (BR1), BR2, and BR3. Air samples were also collected from two 6 x 4 m² housing rooms (HR), HR1 and HR2 (Fig. 1). External atmospheric air samples were used as external control (EC) and were collected from the rodent facility surroundings.

Water samples were collected at specific points as follows: point (1) water supply system (drinking water, DW), (2) post-filtration water (filtered water, FW), and (3) water sterilized by saturated steam under pressure (sterile water, SW). Three samples were collected from each location. One sample was intended for investigation of the presence of bacteria; the second sample was intended for investigation of the presence of yeasts; and the third sample was intended for investigation of filamentous fungi.

All collections were performed in triplicate, once a month, for a total of three months. A total of 324 air samples and 81 water samples were obtained.

Water microbial evaluation

The water microbial content was evaluated by using the total count of bacteria and fungi per milliliter according to the methodology recommended by the "*American Public Health Association*" (APHA 2012). To evaluate the presence of bacteria, 500 mL of water was collected from each collection point into sterile flasks and subsequently filtered by using a 0.22-μm pore membrane (Millipore®). The membrane was placed in a Petri dish containing (i) Reasoner's 2A agar (R2A, Difco, Detroit, MI, USA) for determining the total count of aerobic bacteria, (ii) mannitol agar (MA, Difco) for recovering *Staphylococcus* spp., (iii) cetrimide agar (CA, Difco) for determining *Pseudomonas* spp., and (iv) MacConkey Agar (MCA, Difco) for determining enterobacteria. The Petri dishes were incubated in a bacterial incubator at 37 °C for 24 h. After incubation, the colony forming units per milliliter (CFU/mL) was determined (R2A).

The presence of yeasts was evaluated by collecting 1000 mL of water from each collection point and filtering it through a 0.45-μm pore membrane (Millipore®). The membrane was placed in a Petri dish containing Sabouraud

dextrose agar (SDA, (Difco) supplemented with chloramphenicol (30 mg/100 mL) (Sigma, St. Louis, MO, USA). The dishes were incubated in a biochemical oxygen demand (BOD) incubator at 30 °C for 48 h for fungal growth and subsequent CFU/mL enumeration. Then, they were re-incubated for seven days to verify yeast development.

The presence of filamentous fungi was evaluated in a similar way to the analysis carried out for yeasts; the only difference was the incubation time. After filtration, the dishes were incubated in a BOD incubator for two to three weeks. They were examined from the second day, to verify filamentous fungus development. The samples obtained from point 1 (water supply network) were treated with 1 mL of 1.8% sodium thiosulfate per liter to remove residual chlorine (American Public Health Association, APHA 2012).

Air microbial assessment

The air microbial quality was evaluated by using the total count of bacteria and fungi per cubic meter. Briefly, a one-stage air sampler (MAS-100, Merck KGaA, Darmstadt, Germany) was placed in the center of all the five evaluated rooms (BR1, BR2, BR3, HR1, and HR2) at a height of approximately 1.5 m from the ground, and the sampler was programmed to collect 500 L of air for 5 min after the air impacted the Petri dishes (90 mm) given that the device allows particles with diameter greater than 1 μm to be aspirated.

Tryptic soy agar (TSA, Difco) was used for determining the total count of aerobic bacteria; MA (Difco) was used for recovering *Staphylococcus* spp.; and CA (Difco) and MCA (Difco) were used for determining *Pseudomonas* spp. and enterobacteria, respectively. After the dishes were incubated in a bacterial incubator at 37 °C for 24 h, CFU/m^3 was determined by using the conversion table of the employed device, as recommended by the manufacturer.

The air collection procedure for determining the presence of fungi in air was the same procedure that was used to collect bacteria, except that SDA (Difco) supplemented with chloramphenicol (30 mg/100 mL) (Sigma) was used. For yeasts, the dishes were incubated in a BOD incubator at 30 °C for 48 h for fungal growth and subsequent enumeration of CFU/m^3. Next, they were re-incubated for seven days to verify yeast development. For filamentous fungi, the dishes were incubated in BOD for two to three weeks. They were examined from the second day, to verify filamentous fungus development. The conversion table of the employed device was used to determine the CFU/m^3.

Isolated microorganism identification

The bacterial and yeast strains found in the air and water samples were isolated and later identified by using matrix-assisted laser desorption-ionization-time of flight mass spectrometry (MALDI-TOF MS). Briefly, the microbial culture was suspended in 300 μL of distilled water, to which 900 μL of 99.5% alcohol was added, and centrifuged at 13,000 rpm for 2 min. After centrifugation, the supernatant was discarded; 20 μL of 70% formic acid was added; and the solution was vortexed. After vortexing, 20 μL of acetonitrile was added; and the mixture was centrifuged at 13,000 rpm for 2 min. Then, aliquots of the supernatant were analyzed by mass spectrometry (MALDI-TOF, Bruker MALDI Biotyper 4.0). The criteria established for identification were ≥ 2.0 for species and ≤ 1.7 for genera (Tarumoto et al. 2016).

The isolated filamentous fungi were identified on the basis of the morphological observation of the colony by using giant and microscopic colony techniques, as well as microculture on potato agar (Difco) (Campbell

et al. 2013, Larone 2011, Samson et al. 2007, Silva et al. 2011).

Statistical analysis

Statistical analyses were based on microbial count (CFU/mL). All the results are expressed as the mean ± standard deviation (SD) of the three collections. The data were analyzed by using the Jamovi software (version 2.0) and R: A Language and environment for statistical computing (version 4.0). The obtained data were analyzed by negative binomial regression followed by post-hoc comparisons of groups; Bonferroni corrections were used. Values of p lower than 0.05 were considered statistically significant, with the level of significance set at α = 0.05.

RESULTS

Figure 2 shows the results of quantitative microorganism evaluation in water. The means of the number of bacteria present in DW, FW, and SW ranged from 0.223 ± 0.05 to 0.999 ± 0.91 CFU/mL, whereas the means of the number of fungi present in DW, FW, and SW ranged from 0.015 ± 0.02 to 0.147 ± 0.21 CFU/mL. No statistical difference (α = 0.05) was found for bacteria and fungi regarding the microorganism count in DW, FW, and SW (Table I).

Figure 3 presents the results of the quantitative microorganism evaluation in air. The means of the number of bacteria present in air (Figure 3a) were 132.00 ± 99.0, 130.11 ± 77.0, and 351.56 ± 158.2 CFU/m³ in BR1, BR2, and BR3, respectively, and 51.44 ± 15.01 and 41.00 ± 10.5 CFU/m³ in HR1 and HR2, respectively. The means of the number of fungi in the air of BR1, BR2, BR3, HR1, and HR2 were 12.56 ± 8.9, 12.78 ± 8.6, 36.33 ± 25.8, 10.78 ± 7.7, and 9.11 ± 4.6 CFU/m³ respectively (Figure 3b). In EC, 72.11 ± 10.04 CFU/m³ bacteria (Figure 3a) and 13.11 ± 4.47 CFU/m³ fungi (Figure 3b) were detected.

Statistical analysis of the microbial counts showed that more bacteria than fungi were found in the animal facility air (p = 0.001). The microorganism count in the air of the three breeding rooms (BR1, BR2, and BR3) was higher as compared to rooms HR1 and HR2 (p < 0.05), with BR3 having the highest count (p < 0.05). Compared to EC, BR3 (p = 0.001), HR1 (p = 0.049), and HR2 (p = 0.001) had higher microorganism count (Table II).

A total of 44 species, including bacteria, yeasts, and filamentous fungi, were isolated and

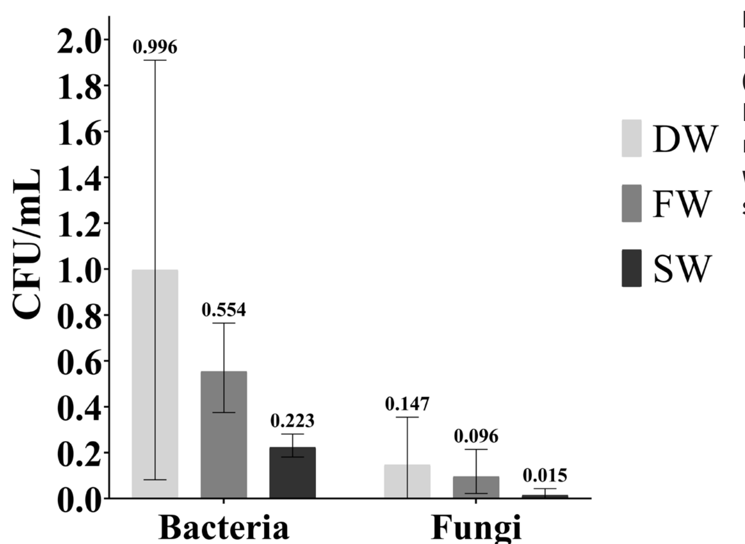

Figure 2. Data presented as the mean of colony forming units (CFU) per milliliter of waterborne bacteria and fungi found in the rodent facility. DW, drinking water; FW, filtered water; SW, sterile water.

identified (Table III). Among these species, 14, 20, and 10 occurred in water samples only, air samples only, and both water and air samples, respectively. Fifty-three microorganisms were found in water; 79 were found in air. Among all the detected microorganisms, eight are considered pathogenic for humans and animals, namely *Aspergillus fumigatus* (detected in DW, FW, BR1, and BR2), *Aspergillus* spp. (detected in FW, SW, BR2, BR3, and HR1), *Corynebacterium* spp. (detected in FW), *Enterobacter cloacae* (detected in DW, FW, and BR2), *Escherichia coli* (detected in SW), *Fusarium* spp. (detected in DW), *Staphylococcus aureus* (detected in DW, FW, and BR1), and *Staphylococcus epidermidis* (detected in FW and SW).

DISCUSSION

We analyzed the presence of microorganisms in water collected at three different points of the animal facility for rodents: DW, FW, and SW. Because the Brazilian legislation has not set specific standards for water microbial quality in animal facilities, we considered the legal criteria defining the control and surveillance of water quality for human consumption and its standard of potability when we analyzed the data for drinking water obtained from the water supply system (Brasil 2011). Owing to the biomedical character of the facility, we employed the Brazilian Pharmacopoeia criteria when we analyzed FW and SW (Agência Nacional de Vigilância Sanitária, ANVISA 2019).

In the case of DW, quantitative analysis detected 0.996 ± 0.91 CFU/mL bacteria and 0.147 ± 0.20 CFU/mL fungi. The Brazilian legislation for DW uses the absence of fecal coliform markers as a parameter for water microbial analysis (Brasil 2011), so we were not able to determine whether the quantitative data obtained herein are in accordance with normality. Although we did not use any specific method for detecting the presence of fecal coliforms in this analysis, we did not detect fecal coliforms in any DW sample when we identified the microorganisms by MALDI-TOF.

We detected 0.554 ± 0.19 CFU/mL bacteria and 0.096 ± 0.10 CFU/mL fungi in FW and 0.223 ± 0.05 CFU/mL bacteria and 0.015 ± 0.02 CFU/mL fungi in SW. According to the Brazilian Pharmacopoeia, FW and SW are considered purified water (produced from DW, without addition of any substance), so we used the recommended monitoring value in which the total bacterial count is ≤ 100 CFU/mL (ANVISA 2019). Therefore, the data we obtained for FW and SW lay within the expected normal standard. None of the Brazilian regulations used in this study enabled us to determine whether the data regarding the quantitative evaluation of fungi present in water were in accordance with normal standard values.

Albeit present in small quantities allowed by the Brazilian legislation (Brasil 2011, ANVISA 2019), we isolated and identified 24 microorganisms in the water samples (Table III). Eight of these microorganisms, namely *Aspergillus fumigatus* (found in DW and FW), *Aspergillus* spp. (found

Table I. Negative binomial regression analysis of microorganism counts from water collections.

Comparisons		exp(B)	SE	z	p
Bacteria	Fungi	1.18e-4	40141.90	-2.25e-4	1.000
DW	FW	0.832	32138.20	-5.71e-6	1.000
DW	SW	9.86e-6	39988.18	-2.88e-4	1.000

in FW and SW), *Corynebacterium* spp. (found in FW), *Enterobacter cloacae* (found in DW and FW), *Escherichia coli* (found in SW), *Fusarium* spp. (found in DW), *Staphylococcus aureus* (found in DW and FW), and *S. epidermidis* (found in SW and FW), have clinical importance for humans and animals (Quinn et al. 2001, Hirsh & Zee 2003, Carroll et al. 2019).

Raynor et al. (1984) evaluated the FW of an animal facility used for scientific research and identified three bacteria: *Delftia acidovorans* (*Pseudomonas acidovorans*), *Achromobacter* spp,. and *Cupriavidus pauculus* (CDC Group IV C-2). Here, we also found *D. acidovorans* in the FW of the rodent facility.

There is no specific Brazilian legislation for the analysis of air microbial quality in animal facilities, either. Therefore, we employed two criteria defined by the Brazilian Health Surveillance Agency (ANVISA). The first criterion establishes the reference standard of indoor air quality in artificially air-conditioned environments for public and collective use and standardizes the maximum recommended values for fungal presence in air (Brasil 2003). The second criterion is applicable for biomedical facilities and acts as a guide for monitoring air quality in the pharmaceutical industry; this criteria is used for classifying clean areas on the basis of the maximum limit of microorganisms in air (ANVISA 2013).

According to the ANVISA criteria for indoor air quality, the maximum recommended value for fungi is ≤ 750 CFU/m³ for an I/E ratio ≤ 1.5, where I represents the indoor environment and E the external control (Brasil 2003). We found less than 750 CFU/m³ fungi in all the five evaluated rooms, which met the ANVISA criteria. However, the I/E ratio in BR3 was 2.7, which is higher than the allowed I/E ratio. The I/E ratio was lower than 1.5 in the other four rooms (BR1, BR2, HR1, and HR2) (Fig. 2b).

Comparison between BR3 and EC ($p = 0.001$) corroborated the I/E ratio result. Nevertheless, multiple comparisons also found increased microorganism count in HR1 and HR2 compared to EC ($p = 0.049$ and 0.001, respectively) and, when the I/E ratio values of these rooms were calculated, they were within the recommended values. This can be explained by exp(B) BR3 3.403 HR1 0.722 HR2 0.62. The rate of microorganism incidence in BR3 was 3.403 times higher than in

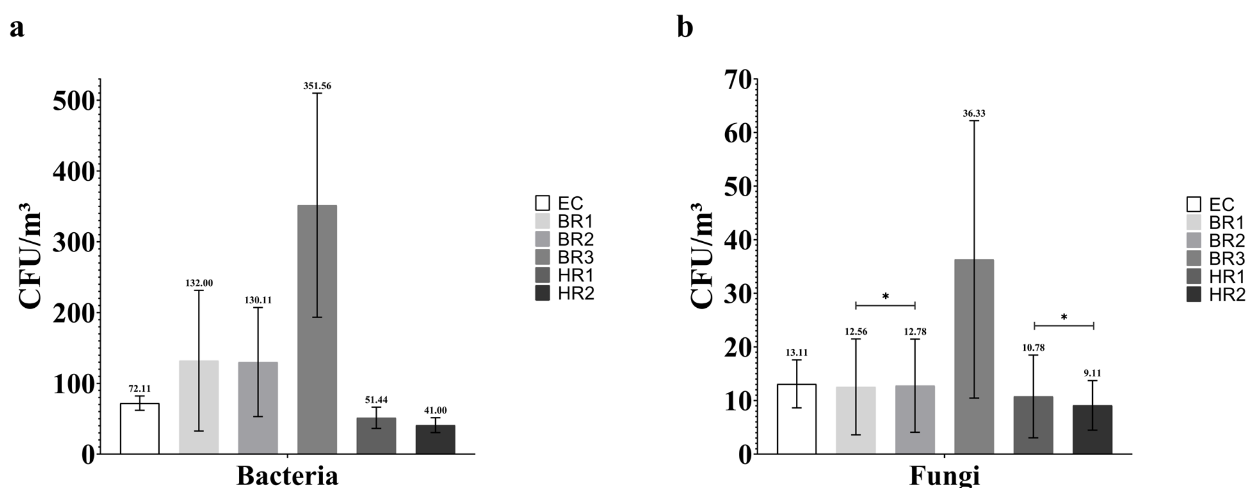

Figure 3. (a) Data presented as mean of colony forming units (CFU) per cubic meter of airborne bacteria found in the rodent facility. (b) Data presented as mean of CFU per cubic meter of airborne fungi found in the rodent facility. EC, external control; BR1, breeding room 1; BR2, breeding room 2. BR3, breeding room 3; HR1, housing room 1; HR2, housing room 2. *Indoor/external ratio (I/E) = ≤ 1.5 CFU/m³.

EC; the rate of microorganism incidence in HR1 and HR2 was 0.722 and 0.623 times smaller than in EC, respectively.

Identification of pathogenic fungi such as *Aspergillus* sp. in air is also outside the standard recommended by ANVISA (Brasil 2003). Here, we isolated and identified four *Aspergillus* sp. strains in the rodent facility (Table III), namely *A. clavatus* (found in BR1 and BR2), *A. flavus* (found in BR1, BR2, HR1, and HR2), *A. fumigatus* (found in BR1 and BR2), and *Aspergillus* spp. (found in BR2, BR3, and HR1). In the EC samples, we detected *A. flavus* (also found in the rooms described above) and *A. niger* (not found in the rooms of the rodent facility) strains. These findings suggested that the external environment does not contribute to *Aspergillus* spp. contamination within the rodent facility.

We isolated and identified the fungi *Paecilomyces variotii* in the air of rooms BR2 and BR3. *P. variotii* is pathogenic for humans (Carroll et al. 2019) and opportunistic for animals (Quinn et al. 2001, Hirsh & Zee 2003). Kunstyr et al. (1997) reported *P. variotii* in the internal organs of animals, including rodents, used for scientific research.

Literature reviews show that Brazil and other countries employ the same parameter for defining the presence of fungi as an air quality marker. However, consensus about the maximum values of fungi allowed in indoor environments is lacking; some guidelines also emphasize assessing the presence of bacteria (Kim et al. 2018, Rao et al. 1996). We isolated and identified 22 bacterial species in the air samples (Table III). Six of these bacteria, namely *Acinetobacter* spp. (BR3), *Bacillus cereus* (BR1, BR2, and HR2), *B. pumilus* (BR1, BR3, HR1, and HR2), *E. cloacae* (BR2), *Serratia marcescens* (BR2), and *S. aureus* (BR1), are potentially pathogenic and opportunistic for humans and animals and have clinical significance. These findings suggested

Table II. Analysis of multiple post hoc Bonferroni comparisons of microorganism counts from air samples.

Comparisons		exp(B)	SE	z	p
Bacteria	Fungi	7.27	0.459	31.4	0.001
BR1	BR2	0.944	0.1058	-0.517	1.000
BR1	BR3	0.317	0.0325	-11.218	0.001
BR1	HR1	1.496	0.1705	3.534	0.006
BR1	HR2	1.734	0.1999	4.774	0.001
BR1	EC	1.080	0.1184	0.701	1.000
BR2	BR3	0.336	0.0341	-10.732	0.001
BR2	HR1	1.585	0.1790	4.081	0.001
BR2	HR2	1.837	0.2103	5.315	0.001
BR2	EC	1.144	0.1246	1.238	1.000
BR3	HR1	4.714	0.4880	14.976	0.001
BR3	HR2	5.464	0.5748	16.142	0.001
BR3	EC	3.403	0.3368	12.372	0.001
HR1	HR2	1.159	0.1349	1.269	1.000
HR1	EC	0.722	0.0800	-2.941	0.049
HR2	EC	0.623	0.0700	-4.215	0.001

that airborne bacterial contamination is a potential health hazard for humans and rodents.

Microbial contamination limit is one of the criteria used by ANVISA for classifying clean areas into grades A, B, C, and D in the pharmaceutical industry. This limit is less than 1 CFU/m³ for grade A, 10 CFU/m³ for grade B, 100 CFU/m³ for grade C, and 200 CFU/m³ for grade D (ANVISA 2013). According to these values and the mean results obtained here, rooms BR1, BR2, and BR3 of the rodent facility can be classified as grade D clean area, while rooms HR1 and HR2 can be classified as grade C clean area.

Here, we detected *Pseudomonas* spp. and *P. nitroreducens* in DW and SW, and *P. oryzihabitans* (BR2), *P. putida* (HR2), and *P. stutzeri* (BR2) in the air samples. We found *S. aureus* strains in DW and FW as well as the air samples collected from BR2. We also found a *Corynebacterium* spp. strain in FW. Our findings agree with an Argentinian study that monitored microbial contamination in the blood and internal organs of rats and mice in a facility used for scientific research from 2012 to 2016. The latter study identified *Proteus* spp. strains and *Pseudomonas aeruginosa*. During the study period, the authors did not detect any *S. aureus* strain, but they detected *Corynebacterium kutscheri* in 12.97% mice and 21.54% rats (Carriquiriborde et al. 2020).

The Federation of European Laboratory Animal Science Associations (FELASA) recommends that the animal health monitoring program for rodents should investigate the presence of certain microorganisms, including *S. aureus* and *C. kutscheri (Felasa 2014)*. As mentioned above, here we detected *S. aureus* and *Corynebacterium* spp. in the water samples. Although we did not monitor rodents, the presence of these bacteria in water represents high risk of infection.

During an immunological study in mice, Mayeux et al. (1995) discovered *A. fumigatus* contamination in animals, which prevented them from conducting the study. Later, the authors tracked the source of fungal contamination to the employed bedding; furthermore, they found 80 CFU/g *Rhodotorula* sp., a yeast, in rodent chow. Here, we found *A. fumigatus* in air samples (BR1 and BR2) and water samples (DW and FW) of the rodent facility. Additionally, we detected other *Aspergillus* spp. in water, namely *Aspergillus* spp. (FW and SW) and *Aspergillus terreus* (DW). We also identified three *Rhodotorula mucilaginosa* strains in DW.

Among the 44 species identified herein, only one is non-pathogenic or opportunistic for humans or animals. *Bacillus atrophaeus*, found in the air of BR2 (Table III), is a spore-forming bacterium that is widely used in biotechnological processes, mainly as a biological indicator of disinfection and sterilization processes (Sella et al. 2015). We detected two microorganisms, *A. niger* and *Lysinibacillus boronitolerans*, only in the air outside the facility. Therefore, in the water and air inside the rodent facility, we identified 41 species of clinically important microorganisms that are pathogenic or opportunistic microorganisms capable of causing diseases in humans and animals.

Accurate and reproducible data are the cornerstone of scientific research. Animals are often used to obtain data, which is vital for research. The immune system of animals maintained under laboratory conditions is sometimes compromised, which renders them susceptible to pathogenic and opportunistic microorganisms. Infected animals may change the results and affect scientific research (Mansfield et al. 2010).

Although the Brazilian legislation has parameters that define the microbial quality of water for human consumption, similar legislation for animal facilities is lacking. Currently, advice regarding indoor air in climate-controlled

Table III. **Microorganisms isolated and identified in the air and water of the rodent facility and their possible pathogenicity for humans and animals.**

Numbers of microorganisms isolated in air and water samples											
Microorganisms	**Water**			**Air**						**Pathogenic to humans[1]**	**Pathogenic to animals[2]**
	DW	**FW**	**SW**	**BR1**	**BR2**	**BR3**	**HR1**	**HR2**	**EC**		
Acinetobacter spp.	0	0	0	0	0	1	0	0	0	Y	O
Aspergillus clavatus	0	0	0	1	1	0	0	0	0	O	O
Aspergillus flavus	0	0	0	1	1	0	1	1	1	Y	O
Aspergillus fumigatus	1	1	0	2	2	0	0	0	0	Y	Y
Aspergillus niger	0	0	0	0	0	0	0	0	2	Y	O
Aspergillus spp.	0	1	2	0	3	2	1	0	0	Y	Y
Aspergillus terreus	1	0	0	0	0	0	0	0	0	Y	O
Bacillus atrophaeus	0	0	0	0	1	0	0	0	0	N	N
Bacillus cereus	2	1	2	1	1	0	0	1	1	O	O
Bacillus circulans	0	0	1	0	0	0	0	0	0	Y	O
Bacillus megaterium	0	0	1	0	0	2	0	0	0	Y	N
Bacillus pumilus	1	2	0	2	0	1	1	1	1	Y	O
Bacillus thuringiensis	0	0	1	0	0	0	0	0	1	O	N
Brevibacillus borstelensis	0	0	1	0	0	0	0	0	0	O	N
Candida parapsilosis	1	0	0	0	0	0	0	0	0	Y	O
Corynebacterium spp.	0	1	0	0	0	0	0	0	0	Y	Y
Delftia acidovorans	0	1	0	0	0	0	0	0	0	O	N
Enterobacter asburiae	0	1	1	0	0	0	0	0	0	O	O
Enterobacter cloacae	2	2	0	0	1	0	0	0	0	Y	Y
Escherichia coli	0	0	1	0	0	0	0	0	0	Y	Y
Fusarium spp.	1	0	0	0	0	0	0	0	0	Y	Y
Lysinibacillus boronitolerans	0	0	0	0	0	0	0	0	1	O	N
Lysinibacillus sphaericus	0	0	0	0	0	0	0	2	0	O	N
Paecilomyces variotii	0	0	0	0	1	1	0	0	0	Y	O
Pantoea eucrina	0	0	0	1	0	1	0	0	1	O	N
Pantoea septica	0	0	0	0	0	0	0	1	0	O	N

Table III. Continuation.

Microorganisms	Water			Air						Pathogenic to humans¹	Pathogenic to animals²
	DW	FW	SW	BR1	BR2	BR3	HR1	HR2	EC		
Pseudomonas nitroreducens	1	0	1	0	0	0	0	0	0	N	N
Pseudomonas oryzihabitans	0	0	0	0	1	0	0	0	0	O	N
Pseudomonas putida	0	0	0	0	0	0	0	1	0	O	O
Pseudomonas stutzeri	0	0	0	0	1	0	0	0	0	O	O
Rhodotorula mucilaginosa	3	0	0	0	0	0	0	0	0	Y	N
Serratia marcescens	1	0	2	0	1	0	0	0	0	Y	O
Staphylococcus aureus	1	3	0	1	0	0	0	0	0	Y	Y
Staphylococcus capitis	1	0	0	0	0	0	0	0	0	O	O
Staphylococcus cohnii	0	0	0	1	0	0	0	0	1	O	O
Staphylococcus epidermidis	0	1	3	0	0	0	0	0	0	Y	Y
Staphylococcus equorum	0	0	0	0	1	0	0	0	0	O	O
Staphylococcus gallinarum	1	2	1	0	0	0	0	0	0	O	O
Staphylococcus lentus	0	1	0	2	2	3	2	2	1	O	O
Staphylococcus nepalensis	0	0	0	1	1	0	1	1	0	O	O
Staphylococcus sciuri	0	0	0	0	1	3	0	2	0	O	O
Staphylococcus succinus	0	0	0	0	0	1	0	0	0	O	O
Staphylococcus warneri	2	0	0	0	1	0	0	0	0	O	O
Staphylococcus xylosus	0	0	0	1	0	0	0	0	2	O	O

Numbers of microorganisms isolated in air and water samples

DW, drinking water; FW, filtered water; SW, sterile water; BR1, breeding room 1; BR2, breeding room 2; BR3, breeding room 3; HR1, housing room 1; HR2, housing room 2; EC, external control. ¹ according to Carrol et al. (2019). ² according to Hirsh & Zee (2003) and Quinn et al. (2001). Y, yes. O, opportunistic. N, no.

environments and recommendations for water and air in the pharmaceutical industry are applied to animal facilities (Brasil 2003, 2011, ANVISA 2013, 2019). However, scientific facilities, such as the rodent facility of the present study, need extra vigilance concerning microbial contamination, and standards and control routines based on their specific requirements must be developed (Politi et al. 2008, Straumfors et al. 2018). In this context, this is the first Brazilian study that has aimed at quantitatively and qualitatively assessing the environmental quality of air and water in a rodent facility used for scientific experimentation.

The identification of potentially pathogenic and opportunistic microorganisms in this study highlights the need for creating monitoring norms and standards for animal experimentation environments. These environments must be reliable and safe for humans, which will prevent

zoonosis and allow reliable scientific results to be generated. This study contributes to the debate on sanitary standards and norms needed for good practices. Such practices will enable researchers to obtain data of desirable quality when they use animals in their experiments and will ensure that employees and users of animal facilities are safe.

CONCLUSIONS

Despite the lack of Brazilian legislation about the microbial quality of water and air in animal facilities, the data obtained here allowed us to conclude that the sanitary standards used by the rodent facility at the Federal University of Uberlândia were effective in maintaining good water microbial quality. However, according to the Brazilian legislation for pharmaceutical industries, the air microbial quality in BR3 did not meet the standards of indoor and climate-controlled environments; the rodent facility rooms BR1, BR2, and BR3 were considered cleaning grade D; and the rodent facility rooms HR1 and HR2 were considered cleaning grade C. A total of 41 microorganisms identified in the water and air of the rodent facility were considered potentially pathogenic or opportunistic for animals and humans. Of the detected microorganisms, *Aspergillus fumigatus*, *Aspergillus* spp., *Corynebacterium* spp., *Enterobacter cloacae*, *Escherichia coli*, *Fusarium* spp., *Staphylococcus aureus*, and *Staphylococcus epidermidis* are pathogenic for humans and animals and can impact the environment, causing problems for rodents and the public health. Further investigations are required to define the sources of air and water contamination in the facility, and specific sanitary standards for these environments should be created, allowing control measures that best suit these facilities to be adopted.

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