Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Analysis of fungal microbiota of ambient air in an intensive care unit in Rio Branco, Acre, Western Amazon, Brazil

Microbiota fúngica do ar ambiente em uma unidade de terapia intensiva em Rio Branco, Acre, Amazônia Ocidental, Brasil

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Abstract

Fungi exhibit three adverse effects on human health: inflammatory, allergic and toxic effects, these implications affect mainly immunodepressed patients. The objective of this work was to analyze the fungal microbiota of the ambient air of an Intensive Care Unit. Three collections were carried out in an Intensive Care Unit in the city of Rio Branco, Acre, Western Amazon, Brazil from March to May 2017. 126 Petri dishes were exposed with the culture medium Agar Sabouraud with chloramphenicol and Agar Mycosel, considering the distribution of the 21 air conditioners, split residential model. The plates were incubated for seven days at room temperature and the growth of Colony Forming Units was observed. Colony counting and isolation for the morphological characterization of the granted fungi was performed. After quantification, the concentration of fungi per cubic meters of air (CFU.m^{-3}) was settled. The third collection had a larger number of colony forming units with 48.6%. In the total of the analyzed samples, filamentous fungi (85.5%) and yeasts (14.5%) were isolated. Thirteen genera of fungi were identified, with the most frequent filaments being *Cladosporium* spp. 33.0%, *Aspergillus* spp. 30.4% and *Penicillium* spp. 19.6%, and yeasts *Candida* spp. 52.6%, *Trichosporon* spp. 36.9%. The colony-forming unit per cubic meter (CFU.m-3) did not shown any difference between the Cores in the same collection period, however in the 1st and 3rd collection, Core 1 had the highest average. The fungal microbiota of this Unit presented thirteen different genera potentially pathogenic, revealing the need for monitoring microorganisms and prevention actions.

Keywords: ICU, fungal infections, yeasts, filamentous fungi, air monitoring.

Resumo

Os fungos exibem três efeitos adversos para a saúde humana: inflamatórios, alérgicos e tóxicos, esses efeitos atingem principalmente pacientes imunodeprimidos. O objetivo do trabalho foi analisar a microbiota fúngica do ar ambiente em uma Unidade de Terapia Intensiva na cidade de Rio Branco, Acre, Amazônia Ocidental, Brasil. Foram realizadas 03 coletas em uma Unidade de Terapia Intensiva na cidade de Rio Branco, Acre, Amazônia Ocidental, Brasil no período de março a maio de 2017. Foram expostas 126 placas de Petri com os meios de cultura Ágar Sabouraud com cloranfenicol e Ágar Mycosel, considerando a distribuição dos 21 condicionadores de ar, modelo split residencial. As placas foram incubadas por sete dias em temperatura ambiente e observado o crescimento das Unidades Formadoras de Colônias. A contagem de colônias e o isolamento para a caracterização morfológica dos fungos isolados foi realizada. Após a quantificação, foi calculada a concentração de fungos por metros cúbicos de ar (UFC.m-3). A terceira coleta teve maior quantidade de unidades formadoras de colônias com 48,6%. No total das amostras analisadas, foram isolados fungos filamentosos (85,5%) e leveduriformes (14,5%). Foram identificados 13 gêneros de fungos, sendo os filamentosos mais frequentes *Cladosporium* spp. 33,0%, *Aspergillus* spp. 30,4% e *Penicillium* spp. 19,6% e os leveduriformes *Candida* spp. 52,6%, *Trichosporon* spp. 36,9%. A unidade formadora de colônia por metros cúbicos (UFC.m-3) não houve diferença entre os Núcleos no mesmo período de coleta, porém na 1ª e 3ª coleta, o Núcleo 1 apresentou maior média. A microbiota fúngica desta Unidade apresentou treze gêneros diferentes potencialmente patogênicos, mostra a necessidade de monitoramento dos microrganismos e ações de prevenção.

Palavras-chave: UTI, infecções fúngicas, leveduras, fungos filamentosos, monitoramento do ar.

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Received: February 24, 2023 – Accepted: August 29, 2023

1. Introduction

Fungi exhibit three adverse effects on human health: inflammatory, allergic and toxic effects (Andrade et al., 2015). These implications mainly affect patients with impaired immune systems and those undergoing treatment in intensive care units (ICU) (Egbuta et al., 2017). Thus, approximately 8% of hospitalized patients have nosocomial infections caused by fungi (Pereira et al., 2014).

Hospital infections caused by fungi are one of the main complications with a direct and significant influence on the patient's recovery. For this reason, there is a need to understand the nature of these microorganisms that cause infections, as in most situations they are treated for long periods with antibiotics, which can develop resistance and lead to death due to the conduct of erroneous or late treatment (Egbuta et al., 2017).

The means of fungal infections can be of endogenous origin, caused by the patient's own microbiota or of exogenous origin, originated from external sources, such as the hands of professionals, invasive devices (catheters, probes, aboucath) and the air conditioning system (Pereira et al., 2014). Among the mentioned means of exogenous fungal infections, there is the air conditioning system that allows the increased risk of contamination through ambient air, revealing a concern regarding the air quality of Intensive Care Units through air conditioners (Egbuta et al., 2017; Pereira et al.,2014).

Fungi are dispersed throughout the environment, and among the various species already described, there are the fungi found suspended in the air called anemophiles (Pereira et al., 2014). And the main genera of anemophilous fungi already described are *Penicillium* spp., *Fusarium* spp., *Acremonium* spp., *Curvularia* spp., *Cladosporium* spp. and *Aspergillus* spp., considered responsible for nosocomial invasive opportunistic infections such as onychomycosis, otitis keratitis, allergic conditions, mycotoxicoses and urinary, respiratory, systemic and disseminated infections (Pereira et al., 2014).

One of the measures to reduce the rate of hospital infections through ambient air in Brazil was the regulation of the ABNT NBR 7256 norm, which establishes the adequate treatment of air in healthcare environments and reference standards for air quality in artificially acclimatized environments, in public and collective use, in order to provide guidance on aspects of air filtration and renewal, as well as operational conditions of the air system such as installation and maintenance (ABNT, 2005).

The high use of air conditioners in health services has been warning about the need to control air quality, as there is little renewal of it, thus requiring a multidisciplinary approach between the most diverse areas of health, because not contemplating this need can influence the length of hospital stay and cause complications to patients through fungal infections (Simões et al., 2011; Brasil, 2004a).

Thus, the description of the fungal presence in ambient air of the Intensive Care Unit is shown to be a fundamental instrument for microbiological control, since air quality can directly and significantly influence the clinical condition and the occurrence of nosocomial infection. of these patients. Therefore, the objective of this study is to analyze the fungal microbiota of ambient air in an Intensive Care Unit in the city of Rio Branco, Acre, Western Amazon, Brazil.

2. Methods

This is a longitudinal study carried out in the Intensive Care Unit (ICU) of the Hospital de Urgência e Emergência de Rio Branco (HUERB), in Rio Branco, Acre. This hospital is state-run, highly complex, a reference unit in the state of Acre and in the borders of the states of Rondônia and Amazonas, and of the countries Peru and Bolivia.

The unit is divided into three centers, one administrative and two assistants (with nine beds each). There are 18 ICU beds, of those, five are in isolation, six are common (Core 1), with the possibility of hemodialysis, and the other seven beds are common (Core 2), totaling an area of 480 m^2. Each Core has one nursing station.

The number of air conditioners found in the unit is one in each bed, one in the warehouse and two central appliances for the center hall. All air conditioners are residential model, as well, the filter is a common air filter screen.

Two collection instruments were applied. These instruments were previously elaborated based on NBR 7256 (ABNT, 2005) and Ordinance MS/GM nº. 3523 of 08/28/98 (Brasil, 1998). The first instrument was completed by the person responsible for the Intensive Care Unit, in just one moment, with the objective of characterizing the unit in the following aspects: physical structure and location of the unit, temperature and humidity control, air conditioner specifications, cleaning and maintenance routine (Simões et al., 2011).

The second instrument, on the other hand, was applied by the researchers, during the three collections performed, recording the physical structure, the cleaning of the units and the air conditioners (Simões et al., 2011).

The Petri dishes were exposed at three different collection times, 0, 30 and 60 days, from March to May 2017. The distribution and exposure of the two dishes was linked to the location of the air conditioner. On one plate Sabouraud Agar was added, plus chloramphenicol and on the other mycosel Agar, totaling 126 plates distributed in 21 places (beds, isolations, warehouse and nursing stations) of the intensive care unit and 12 control, in each collection.

At each exhibition point, the plates remained open for 15 minutes on a stainless steel Mayo table (instrumental), at a height of one meter in relation to the floor and in front of the air conditioner (Pereira et al., 2014; Silva et al., 2017).

All samples were sent and packed in plastic material organizing boxes to the Plant Health Laboratory of the Federal University of Acre (UFAC). These plates were incubated at room temperature and analyzed for seven days (Brasil, 2004a).

After the growth of the colonies, the counting of Colony Forming Units (CFU) was performed for each sampled area with the aid of an electronic colony counter (Lacaz et al., 2002). To purify the fungi, fragments of the colonies were removed and inoculated using the streak technique by depletion in Petri dishes containing Sabouraud Agar supplemented with chloramphenicol or rose Bengal (Lacaz et al., 2002).

After purification, the colonies were transferred to test tubes containing Agar Sabouraud and Potato Dextrose Agar, for preservation and identification of fungi. Then, the inoculum fragment was removed and placed between slide and coverslip with lactophenol cotton blue to visualize the fungal structures (Riddell, 1950).

The identifications were performed by macro and micromorphological analysis of the colonies visualized under an optical microscope, with a 40-fold magnification. When necessary for better visualization of the microstructures, the slide culture method was used (Riddell, 1950). The identification of the genera was done using the identification keys Barnett and Hunter (1998), Hoog and Guarro (1995), Raper and Fennell (1977) and Ellis (1976).

After incubation, the concentration of fungi per cubic meters of air (CFU.m-3) was calculated according to Omelyansky (1940) using the following formula:

$N = 5a \times 10^4 (bt)^{-1}$, in which N= CFU.$m^{-3}$ of air, a= number of colonies per petri dish, b= plate area in cm^2, t= exposure time in minutes.

For descriptive analysis, absolute and relative frequencies, mean and standard deviation were used. The results were tabulated in the Microsoft Excel® 2013 program. For inferential statistical analysis, the following tests were applied: Shapiro-Wilk Normality test; Bartlett's test of Homogeneity of Variances; Student's t-test comparison of means; Kruskal-Wallis with Dunns post-test. Inferential analysis was performed using the GraphPad Prism® 8.0.2 program, considering it significant when $p < 0.05$.

This study was approved by the Research Ethics Committee of the Fundação Hospitalar Estadual do Acre (FUNDHACRE) by CAAE nº 47577215.2.0000.5009.

3. Results

The results of the researcher's observations during the three collections identified problems in the maintenance and physical structure such as visible signs of infiltration, corrosion in the air conditioner cabinet, and cracks in the walls.

Table 1. CFU growth percentage in the first, second and third collection at the Intensive Care Unit, Rio Branco, Acre, Brazil, 2017.

Collections	Colony Forming Units	
	CFU	%
First collection	184	36.40
Second collection	76	15.00
Third collection	246	48.60
Mean/Standard deviation	168.7(±49.7)	-
Total	**506**	**100**

CFU: Colony forming units.

The technician responsible for the unit mentioned the existence of occupant control; however, there is currently no equipment in the unit to check the humidity and temperature.

The air conditioner inlet faces the front street where one of the hospital's entrances is located, and it is relevant to note that *Columba livia* (pigeons) surround the area.

The maintenance and control of air conditioners is carried out by an outsourced company. The filters are cleaned monthly but not disinfected, and there is no record related to their exchange. In the air conditioner cabinet, cleaning is performed according to the necessities.

The terminal cleaning of the unit is done weekly including general washing of the walls, glass, floor, and curtains, and the beds are also cleaned upon discharge, death, or exchange of patients.

With this panorama of the structural and operational aspects of the Intensive Care Unit described, the results related to fungal CFUs, during the three collections performed, are described in Table 1, where the first and third collections are above average.

Filamentous and yeast-like fungi were identified, with a significantly increased frequency of filamentous fungi in the three collections, as shown in Table 2.

A total of 131 colonies of anemophilic fungi were isolated, with 112 filamentous and 19 yeast colonies, including 13 genera and 10 filamentous fungi. The most frequent genera were *Cladosporium* (33%), *Aspergillus* (30.4%), and *Penicillium* (19.6%). Among the yeasts, three genera were identified, with a higher percentage of *Candida* (52.6%), *Trichosporon* (36.9%), and *Rhodotorula* (10.5%) (Figure 1).

There was no growth observed in the fungal colonies on the 12 control plates that remained throughout the study period.

As shown in Table 3, common beds had the highest percentage of identified genera and the only genera found in all sectors and in the three collections was *Cladosporium* spp.

Regarding the places of exposure of the plates, there was no statistical significance between the average number of colonies from the common beds (6.5) and the isolation beds (6.6) that were investigated. However, this analysis was not conducted for the nursing station and warehouse, as these locations had an average of less than three colonies (Table 3).

Regarding the CFU per cubic meter (CFU.m^{-3}) in Table 4, there was no difference between the Cores in the same collection period; however, in the first and third collections, Core 1 had the highest average, although this was not significant.

Table 2. Percentage of filamentous fungi and yeasts identified in the first, second and third collection in an Intensive Care Unit, Rio Branco, Acre, Brazil, 2017.

Anemophilous Fungi	Collections								
	First		Second		Third		Total		$\bar{x} \pm \sigma$
	N	%	N	%	N	%	N	%	
Filamentous	38	84.4	33	76.7	41	95.4	112	85.5	37.3 (± 2.3) ***
Yeast	7	15.6	10	23.3	2	4.6	19	14.5	6.3 (± 2.3) ***
Total	**45**	**100**	**43**	**100**	**43**	**100**	**131**	**100**	**43.7 (±0.7)**

*** (p< 0,001); N: Number of colonies, $\bar{x}$ = Mean, σ = Standard deviation.

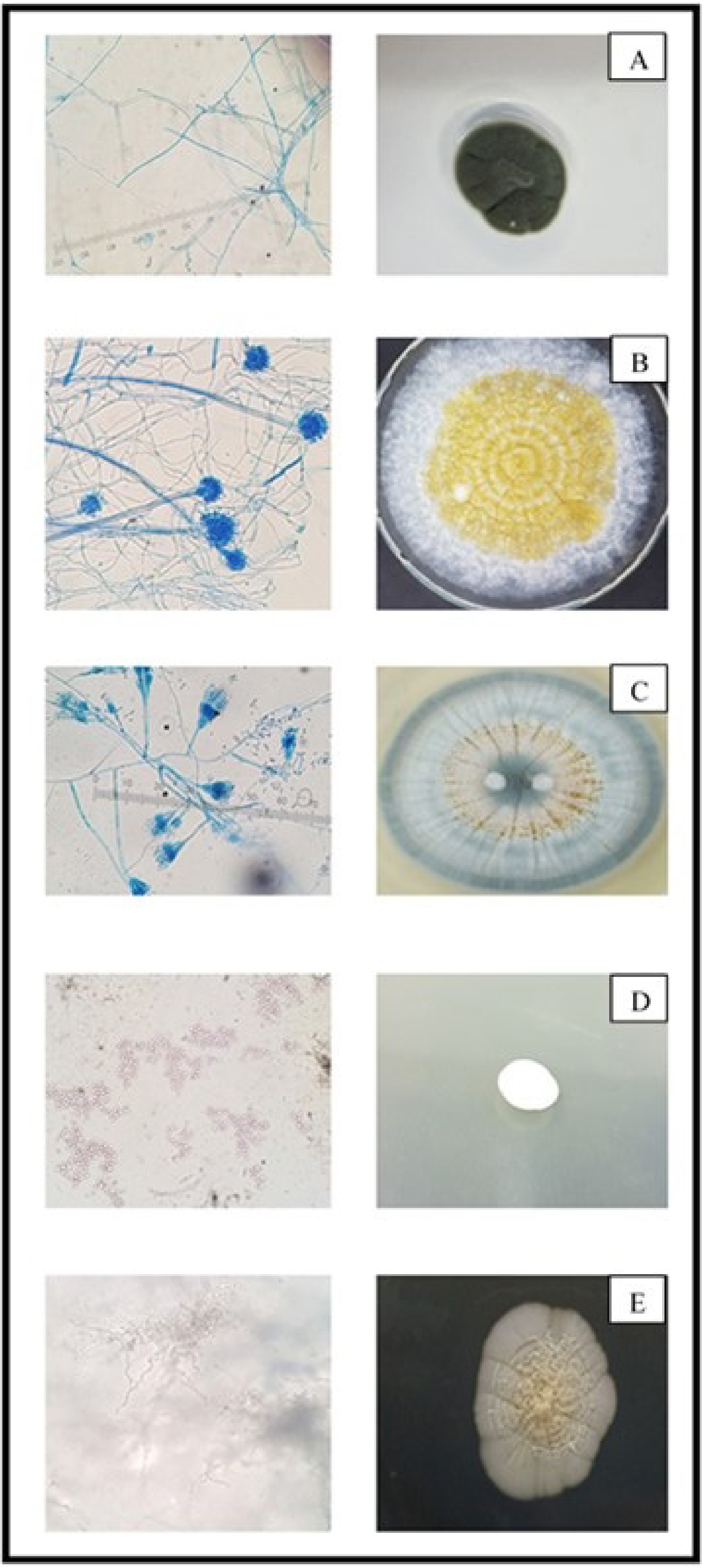

Figure 1. Microscopic and macroscopic of the most frequent fungal genera isolated in the Intensive Care Unit, Rio Branco, Acre, Brazil, 2017. A) *Cladosporium*, B) *Aspergillus*, C) *Penicillium*, D) *Candida*, E) *Trichosporon*.

Table 3. Presence of anemophilic fungal genera by sectors collected in an intensive care unit, Rio Branco, Acre, Brazil, 2017.

Genera	Common bed (CS:13)			Isolation (CS: 05)			Nursing Station (CS: 02)			Warehouse (CS: 01)			Total (CS: 21)
	N	$\overline{x}$	%	N	$\overline{x}$	%	N	$\overline{x}$	%	N	$\overline{x}$	%	N
Cladosporium spp.	26	2	70.3	7	1.4	18.9	3	0.67	8.1	1	1	2.7	37
Aspergillus spp.	22	1.7	64.7	9	1.8	26.5	3	0.67	8.8	0	0	0	34
Penicillius spp.	16	1.2	72.7	6	1.2	27.2	0	0	0	0	0	0	22
Candida spp.	6	0.5	60	2	0.4	20	2	1	20	0	0	0	10
Trichosporon spp.	2	0.15	28.6	4	0.8	57.1	1	0.5	14.3	0	0	0	7
Trichoderma spp.	2	0.15	40	1	0.2	20	2	1	40	0	0	0	5
Mucor spp.	4	0.3	80	1	0.2	20	0	0	0	0	0	0	5
Paecilomyces spp.	2	0.15	50	2	0.4	50	0	0	0	0	0	0	4
Fusarium spp.	2	0.15	100	0	0	0	0	0	0	0	0	0	2
Rhodotorula spp.	1	0.08	50	1	0.2	50	0	0	0	0	0	0	2
Curvularia spp	0	0	0	0	0	0	0	0	0	1	1	100	1
Pestalotiopsis spp	0	0	0	0	0	0	1	0.5	100	0	0	0	1
Cunninghamella spp.	1	0.08	100	0	0	0	0	0	0	0	0	0	1
Total	**84**	**6.5**	**64.1**	**33**	**6.6**	**25.2**	**12**	**6**	**9.2**	**2**	**2**	**1.5**	**131**

CS: Collection Sites, N: number of colonies; $\overline{x}$: Mean.

Table 4. Fungal distribution (CFU.m^{-3}) by Cores collected in an Intensive Care Unit, Rio Branco, Acre, Brazil 2017.

Sector	Collection ($\overline{x}$ ± σ)		
	1st Collection	2nd Collection	3rd Collection
Core 1	412.62 ±536.10Aa	152.30 ± 216.10Aa	437.18 ± 600.40Aa
Core 2	114.62 ±90.18Aa	72.05 +-64.42Aa	340.60 ± 514.30Aa
Isolation	377.3 ±613.10Aa	165.1 ±105.40Aa	306.50 ±44.70Aa
Warehouse	29.47	88.42	117.89

$\overline{x}$ = Mean, σ = standard deviation. Same letters from the same column, demonstrate that there was no statistical significance in the comparison between the Sectors in each collection (Kruskal-Wallis, p> 0.05); Equal letters of the same line, demonstrate that there was no statistical significance in the comparison between the collections of the same Sector (Kruskal-Wallis, p> 0.05).

There was no difference between the CFU.m^{-3} of the same Core between the different collections, but it is noteworthy that the second collection had the lowest averages; there may be due to environmental interference in the values and interference caused by the air conditioners getting cleaned on the day of this collection (Table 4).

4. Discussion

The ICU of the city of Rio de Branco, Acre had damage to its physical structure and lacked equipment for humidity and temperature control.

In the three collections performed over 60 days, a total of 506 CFUs were isolated in Acre, 184 (36.4%) in the first collection, 76 (15%) in the second collection, and 246 (48.6%) in the third collection.

Thirteen genera of fungi were identified, with the most frequent filamentous genera being *Cladosporium* spp. (33.0%), *Aspergillus* spp. (30.4%), and *Penicillium* spp. (19.6%), followed by *Candida* spp. (52.6%) and *Trichosporon* spp. (36.9%). All of these genera have species described as pathogenic to humans, thus emphasizing the importance of microbial control measures in the ICU.

Hospital infections of fungal origin can cause serious complications in patients, contributing to an increase in the rate of morbidity and mortality since the diagnosis and treatment of these infections are challenging. This raises a concern regarding the indoor air quality in hospital environments, such as in ICUs, due to the presence of anemophilic fungi (Skiada et al., 2017).

Pereira et al. (2014) collected a sample of anemophilic fungi from various sectors of a hospital and found 12 genera of filamentous and yeast-like fungi, which is similar to the present study wherein 13 genera were identified in the ICU.

Silva et al (2017) showed that closed environments, such as the ICU where there is high traffic of people as they provide assistance to the patient and where High Efficiency Particulate Arrestance (HEPA) filters are not used in air conditioners, are appropriate places for proliferation of fungi, especially those which belong to the genus *Aspergillus*. This was one of the most commonly isolated fungal genera in this study.

Aspergillus spp. have three mechanisms of attack: opportunistic infections, allergic states, and toxicosis, with infection mainly occurring due to the inhalation of spores. This genus has been associated with clinical pictures of chronic granulomatous sinusitis, keratitis, otomycosis, cutaneous aspergillosis, osteomyelitis, and invasive pulmonary aspergillosis (Ghiasian et al., 2016). The severity of infection can vary from hypersensitivity to a fatal invasive lung disease, and the severity of the host's involvement is directly dependent on the immune response to this agent (Silva et al., 2017).

Considering pathogenicity, the fungal genera *Cladosporium*, *Aspergillus*, and *Penicillium,* which were isolated more frequently in the present study, are also reported to predominate in other hospitals (Gonçalves et al., 2018; Andrade et al.,2015).

The predominance of *Penicillium* ssp. may indicate a deficiency in air quality and internal sources of contamination (Calumby et al., 2019). In this study, this genus had a frequency of 19.60% that indicated that this particular organism warrants attention.

Andrade et al (2015) showed that the ICU was the location with the greatest contamination by fungi in a hospital, with the presence of *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. Isolation of yeast-like fungi in the ICU was also observed by Gonçalves et al. (2015), who isolated *Candida* spp. and *Rhodotorulla* spp. genera that were also described in this study.

Systemic fungal infections caused by *Candida* spp. and *Aspergillus* spp. are common in patients undergoing treatment in the ICU as compared to other genera found in this sector (Yew et al., 2014). However, in the present study, the most frequent genus identified in the ambient air was *Cladosporium* spp., and *Candida* spp. was the most frequent yeast species.

Candida spp. is the most prevalent and common cause of nosocomial fungal infections in ICUs (Garbee et al., 2017). There are some risk factors associated with its occurrence, such as the use of immunosuppressants, antibiotics, parenteral nutrition, central venous catheters, and surgery (Patolia et al., 2013). Invasive *Candida* is the most frequent cause of blood infections, and opportunistic infections can spread quickly to the heart, brain, eyes, bones, and other organs (Patolia et al., 2013).

Cladosporium spp. is considered as an allergen that can cause severe disease in the respiratory tract with intrabronchial lesions (Menezes et al., 2017). This fungus has been recognized as a pathogenic and toxigenic agent in humans, which can cause skin lesions, brain abscess, pneumonia, phaeohyphomycosis, and the severity of this infection varies according to the individual's immune system (Menezes et al., 2017). In its systemic form, it mainly affects the lungs and brain (Menezes et al., 2017).

The second most frequent genus of yeast fungi identified in this study was *Trichosporon* spp. This causes superficial mycoses in the scalp, armpit, and pubic regions, as well as other infections such as white piedra, pneumonia, endocarditis, peritonitis, and urinary tract infection (Mattede et al., 2015; Mezzari et al., 2015). The presence of this agent has also been described in immunocompromised patients with hematological malignancies and burns (Skiada et al., 2017).

Among the four sectors analyzed for air quality, most fungi were isolated in the common beds (64.1%), in isolation (25.2%), nursing station (9.2%), and warehouse (1.5%). There was a significant difference observed between the common beds, which authorized persons had free access to, and the isolation beds, which were beds located in a closed space with limited access due to contact precautions and to reduce transmission through aerosols and droplets (Brasil, 2004b).

Resolution nº. 176 establishes reference standards for air quality with periodic recommendations for monitoring, environmental control, cleaning procedures, and maintenance of the components of the artificial air conditioning system, in addition to the structural aspects related to the presence of infiltrations and cracks that favor the proliferation of anemophilic fungi (Brasil, 2000).

Columba livia (pigeons) observed in the vicinity of the hospital is one of the main reservoirs *Cryptococcus* (Mezzari et al. 2015). The fungus *Cryptococcus* spp. is recognized for its pathogenic potential especially in immunocompromised hosts, causing pulmonary infections and disseminated in other organs and system (Mezzari et al., 2015). Although this genus was not found in the ICU ambient air, the presence of these animals in the vicinity of the unit should serve as a warning to the management who should take preventive measures.

In a study carried out in an ICU in Mexico, the average concentration of fungi in the air was 85.08 ± 29.19 CFU.m^{-3}, and the most frequent isolated fungal genera were *Cladosporium* spp. (47.34 CFU.m^{-3}), *Penicillium* spp. (19 CFU.m^{-3}) and *Aspergillus* spp. (8.35 CFU.m^{-3}) (Ríos-Yuil et al., 2012), which is similar to the findings of the present study. However, the occurrence of fungal genera was associated with higher concentrations of CFU.m^{-3}.

The Brazilian Standard ABNT NBR 7256: 2005 determines that ICUs with an artificial air conditioning system must use at least class G3 filters in conjunction with class A3 filters (HEPA) with an efficiency of 80–99.97% (ABNT, 2005). The type of filter is another important aspect for health protection from external polluting sources that must be taken into account, as the HEPA filter is highly efficient in air filtration and this filter was not used in the unit under study (ABNT, 2005). Another measure to reduce the proliferation of fungi is periodic cleaning, both of the air conditioners and of the unit as indicated by the significant reduction in fungal colonies during the second collection.

These protective measures serve to reduce the proliferation of anemophilic fungi in the ICU. Following these measures will help ensure a decrease in the risk of invasive fungal diseases occurring in critically ill as well as in immunocompromised individuals (Calumby et al., 2019; Andrade et al., 2015).

Thus, specific legislation for health environments is required to define parameters according to the specificity of each service unit with regard to the environment as this affects the transmission of infectious processes to susceptible patients, as well as the maintenance of the health of the professionals who work in these units. Thus, fungal microbiological control and evaluation are indispensable.

Thirteen genera were identified among the fungal microbiota in the ambient air of an ICU in the municipality of Rio Branco, Acre. The most frequent genera identified were *Cladosporium*, *Aspergillus*, and *Penicillium*, which have species that are pathogenic to humans and cause complications in the clinical status of patients hospitalized in the ICU.

Acknowledgements

We are also grateful to the Dean of Research and Graduate Studies at the Federal University of Acre (UFAC), the Multiprofessional Residency Program with an emphasis on Intensive Care, the Municipal Health Secretariat of Rio Branco, Acre, Brazil and the State Health Secretariat of the State of Acre, Acre, Brazil. We are grateful to Rejane Vieira Santos, in memoriam, for the incentive and for making the research feasible.

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