

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.812.149>

## Airborne Fungi in Neonatal Intensive Care Unit of a Public Hospital in Brazil

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### ABSTRACT

Fungi are opportunistic organisms with wide geographical distribution and can also be found in the hospital environment. These microorganisms can cause infections, especially in immunocompromised patients. The aim of this study was to evaluate airborne fungal contamination in two neonatal intensive care units (ICU) of a public hospital before and after cleaning. The technique of Petri dishes exposure containing Sabouraud agar with 50mg/L chloramphenicol was used for sample collection. Air conditioning filters were also sampled using a sterile swab for fungal collection. The identification of fungal isolates was performed by observing macroscopic and microscopic structures. A total of 1305 colony forming units was isolated, where: 718 (55.0%) were isolated before neonatal ICU cleaning and 587 (45.0%) after cleaning. Forty-two species belonging to 24 genera were identified, being *Cladosporium cladosporioides*, *Penicillium aurantiogriseum* and *Aspergillus oryzae* the most frequent species in the analyzed samples. The presence of pathogenic fungi in ICUs demonstrates the need for constant monitoring of indoor air quality in order to better control airborne contamination in hospital environments.

#### Keywords

Air quality,  
Airborne fungi,  
Neonatal ICU,  
Hospital infection,  
Indoor air

#### Article Info

Accepted:  
10 November 2019  
Available Online:  
10 December 2019

## Introduction

The fungi spread through the atmospheric air are called airborne fungi and by being opportunistic they can cause human diseases. The airborne fungi are related to human health, mainly by the potential to trigger allergic processes, irritation of mucous membranes and skin and fungal infections in people sensitive to their seedlings and toxigenic metabolites (Baxi *et al.*, 2016; Setlhare *et al.*, 2014).

Opportunistic fungi such those from the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Candida* are responsible for diseases ranging from ear infections, cutaneous infections, mycotoxicosis, urinary infections, pneumonia, onychomycosis, eye infections until fungemias (Nascimento *et al.*, 2019).

Hospital environment often has microorganisms in the air, floor, walls, surgical equipment, hospital furniture, refrigeration systems and employees, preferentially infecting immunocompromised patients who use catheters and dialysis, as well as newborns and elderly, causing severe intra-hospital infections (Cristina *et al.*, 2009; Setlhare *et al.*, 2014). Therefore, indoor air quality procedures in hospital environments are critical factors in preventing infections (Azimi *et al.*, 2013).

Hospital infections are an important factor for increased neonatal mortality. Literature data show that mortality in this age range is between 15.5 and 64.4% in cases related to nosocomial infection (NI).

Infections in newborns are always considered from hospital, except for those transmitted via placenta and those associated with premature rupture of membranes for more than 24 hours (Duarte and Mendonça, 2005). The first

reports about the importance of the hospital environment as a transmission source of infectious agents associated with air pollution analyzed the spores of *Aspergillus* sp, thus, exposure to air contaminated by fungi and its metabolites in indoor air-conditioned have worried the scientific and health community around the world (Boff *et al.*, 2013).

Due to the pathogenic potential of airborne fungi, especially in neonates cared in hospital air-conditioned environments (Cristina *et al.*, 2009), the aim of this study was to evaluate airborne fungal contamination in two neonatal intensive care units (ICU) of a public hospital before and after cleaning.

## Material and Methods

### Site and collection technique

Air samples were collected from two sites (A and B) of climatized neonatal intensive care unit at the university hospital. Dr. Alberto Antunes located in the city of Maceió, Alagoas, Brazil.

Fungi collection from the air was performed by passive sedimentation through Petri dishes exposure containing Sabouraud agar plus 50 mg/L chloramphenicol for deposition of fungal propagules present in atmospheric air according to Napoli *et al.*, (2012). Disposable Petri dishes were opened in both environments during 20 minutes at a height of one meter from the floor. Before and after cleaning, 10 plates were exposed to the environment A and 10 plates in the environment B, totaling 400 exposures during the study.

Sample collection from the air conditioner filters was performed using a sterile swab moistened with sterile water plus 50 mg/L chloramphenicol, subsequently seeded by radial spreading into a disposable Petri dish containing the medium described above.

## Fungi Identification

Filamentous fungi identification was based on macroscopic aspects of colonies and microscopic characteristics by direct examination of the culture according to Hoog *et al.*, (2000). Those which could not be identified by the absence of reproductive structures were grown in special medium to stimulate sporulation, using the in-slides microculture technique according to Ridel (1950). After identification, isolates were preserved under mineral oil according to Sherf (1943).

## Statistical analysis

Results were analyzed in the software Biostat® 5.0 using the paired Anova-Manova, t-test and z-test applied to assess the variables found before and after the neonatal ICU hygiene. A p-value <0.05 was considered statistically significant.

## Results and Discussion

Quantitative results of airborne fungi isolated from Neonatal ICU before and after cleaning are shown in Table 1. Analysis of colonies showed the presence of total 1305 colony forming units (CFU); 718 CFU (55.0%) of these were isolated before cleaning and 587 CFU (45.0%) after cleaning the neonatal ICU. The total average values of colonies found in each sample before and after cleaning were  $8.23 \pm 7.39$  and  $11.19 \pm 10.34$  CFU, respectively.

Overall, 772 CFU were isolated in the environment A, being 400 CFU before and 372 after cleaning, with average value of  $9.07 \pm 12.04$  CFU and  $7.93 \pm 12.95$  CFU respectively, with no reduction in fungi concentration after cleaning (Figure 1). In environment B were isolated 533 CFU, being 318 CFU obtained before and 215 CFU after

cleaning, with average of  $7.38 \pm 10.34$  CFU and  $5.10 \pm 7.96$  respectively (Figure 1). It was observed a statistically significant reduction in CFU values after cleaning only in the environment B.

Table 1 shows the identification of pathogenic, toxigenic and allergenic fungi in both environments (A and B) from the neonatal ICU. During this study, 42 species in 24 genera were identified according to the classification system of Alexopoulos (1996), in the class of Deuteromycetes, subclass Hyphomycetidae, including two families Moniliaceae and Dematiaceae.

Among the genera isolated, *Cladosporium* was the most frequent with 324 (26.8%) CFU, followed by *Penicillium* with 300 CFU (24.8%) and *Aspergillus* with 257 (21.3%); in addition, other genera showed frequencies less than 4.7% of total colonies isolated. The most frequent species observed in this study were *C. cladosporioides* (186 CFU, 15.4%), followed by *P. aurantiogriseum* (132 UFC, 10.9%) and *A. oryzae* (113 CFU, 9.3%). According to fungi characteristics, 27 (65.8%) are considered pathogenic, 9 (21.9%) non-pathogenic, 4 (9.8%) allergenic and 1 (2.5%) toxigenic. All species from the genus *Aspergillus* found in both neonatal ICU environments are pathogenic and among them, *A. oryzae* and *A. fumigatus* are also allergenic. Among *Penicillium* species, only *P. aurantiogriseum* and *P. verruculosum* were not reported as pathogenic to man.

A total of 59 CFU of yeasts were isolated from the air at the neonatal ICU, 26 CFU (44.1%) from the environment A and 33 CFU (55.9%) from the environment B (Table 1). There was no significant CFU reduction after the environment cleanup. Among the yeasts, the species *Candida parapsilosis* was the most frequent with 35 CFU (59.3%), followed by *C. guilliermondii* with 11 CFU (18.6%), *C.*

*albicans* with 7 CFU (11.8%), *C. tropicalis* with 3 UFC (5.1%). Three unidentified yeasts do not amplified with the species-specific primers used.

The airborne fungi analysis before and after cleaning the environments A and B showed CFU reduction for the genus *Cladosporium*. However, the numbers of CFU from the genera *Aspergillus* and *Penicillium* obtained before cleaning were lower than those observed after cleanup completion, observing a significant increase ( $p < 0.05$ ) of these genera (Figure 2).

Figure 3 shows the fungi species isolated from the air conditioner filters. The species *Cladosporium cladosporioides* was isolated in almost 80% of cases, followed by *C. herbarum* with 30%. It is also noteworthy: *C. sphaerospermum* and *P. aurantiogriseum* both with about 20% (Figure 3).

Results of this work and other studies such those developed by Setlhare *et al.*, (2014) and Cabo Verde *et al.*, (2015) in hospital environments show the diversity of fungi capable of developing allergic reactions and sensitization of atopic individuals as well as fungal infections of diverse etiologies. This factor corroborates with the indoors airborne fungi researches monitoring.

The presence of pathogenic fungi is associated with various diseases, among them the aspergillosis; determining specific pathological conditions, especially in immunocompromised patients, neonates and children (Cristina *et al.*, 2009). Furthermore, fungi contamination of the indoor atmosphere may influence the occurrence of invasive aspergillosis in intensive care units (Boff *et al.*, 2013). The isolation of several *Aspergillus* species previously described as pathogenic in this study suggests the possibility of development of various symptoms that can lead to illness, absenteeism of employees, as

well as emerging risk to patients in the ICU. The well-performed cleaning and disinfection in hospitals are effective measures of prevention and extinction of the epidemiological chain of infections (Gebel *et al.*, 2013). However, the cleaning improperly performed cannot eliminate the microorganisms and move the microbial load from one location to another (Vikke and Giebner, 2016). In this sense, it is of concern the number of fungi CFU that increased or remained in the environment A, even after cleaning since fungi are used as epidemiological markers for monitoring of artificially air-conditioned environment by national and international health monitoring agencies.

One of the reasons for not reducing the CFU quantity in the environment A may be the fact that cleaning first occurs in the environment B, thus allowing the passage of fungal spores by scanning and re-using of rags and little concentrated solutions which did not eliminate microorganisms. The increased number of CFU after the hygiene for fungi from the genera *Aspergillus* and *Penicillium* can be justified by the high sporulation produced by these fungi and the inadequate hygiene that allows spores suspension in the environment air. These results support the need of changes in the management of some hospital services to introduce improvements in procedures and technologies of cleaning activity, hygiene and monitoring of hospital air-conditioned environments.

Studies developed by Tong *et al.*, (2017) and Gonçalves *et al.*, (2017) on fungal dispersion and hospital environments indicated that events of disinfection, ventilation and personnel transit are determining factors for the presence of anemophilus fungi in the environment. Of all factors, human presence contributes significantly to the fungal diversity observed in indoor air in hospitals. The *Cladosporium* and *Penicillium* genus are

among the most reported in the literature as air contaminants in hospital environments and our results have corroborated with results from previous studies. (Sepahvand *et al.*, 2017). According to Lobato *et al.*, (2009) the seasonality and prevalence of airborne fungi in a hospital in southern Rio Grande do Sul – Brazil showed a higher frequency of *Cladosporium*, *Aspergillus* and *Alternaria*. However, Rostami *et al.*, (2017) showed the presence of the Yeast species, *Penicillium* and *Aspergillus* as being fungi more prevalent in various sectors of an Educational, Research and Treatment Center.

High microbial counts were found in a hospital in the Free State province - South Africa, the genera of fungi identified included *Candida*, *Aureobasidium*, *Phoma* and *Penicillium*. Some of these microorganisms can cause food spoilage and human diseases, especially in immunocompromised patients (Setlhare *et al.*, 2014). Total mean concentration fungi in the hospital rooms in Tehran - Iran was 55 CFU/m<sup>3</sup>, these concentrations showed different levels of contamination with the highest 97 CFU/m<sup>3</sup> observed in Orthopedics Operating Room. The most common fungi were *Penicillium* (70%), *Aspergillus* (14%), *Cladosporium* (12%) and *Alternaria* (2%) (Azimi *et al.*, 2013).

Analyzes carried out by Okten and Asan (2012) in the indoor and outdoor atmosphere of the Pediatric Unit in a Hospital in Edirne – Turkey showed 65 species of fungi from 16 genera, the most frequent genus was *Cladosporium* (33.58%), *Alternaria* (22.53%) and *Penicillium* (20.35%). The most prevalent airborne fungi contaminating intensive care units and operation rooms in Assiut Hospitals – Egypt were *Cladosporium*, *Aspergillus*, *Penicillium* and *Fusarium* (Aboul-Nasr *et al.*,

2014). A study in Davanagere – India showed the presence of *Aspergillus* spp, *Curvularia* spp, *Alternaria* spp, *Penicillium* spp, *Rhizopus* spp, *Nigrospora* spp and *Fusarium* spp in health care centers (Rangaswamy *et al.*, 2013).

In this study, *Aspergillus* spp was dominant in the Government health care center and *Alternaria* spp and *Curvularia* spp were dominant in the private health care center.

Indoor fungal characterization at different hospital sites in Setúbal – Portugal showed the prevalence of genera *Penicillium* (41%), *Aspergillus* (24%) and *Cladosporium* (14%) (Cabo Verde *et al.*, 2015). On the other hand, Rocha *et al.*, (2012) observed that most of the public hospital sites in Caracas, showed fungal densities values within the “very clean” or “clean” standards. These authors also showed the isolation of 12 genera and 5 species, including *Aspergillus* and *Penicillium* spp. as the most frequent fungi. Chang *et al.*, (2015) also reported that the fungal concentrations for different locations in Intensive Care Unit in Taiwan did not reach statistical significance and generally were in the standards.

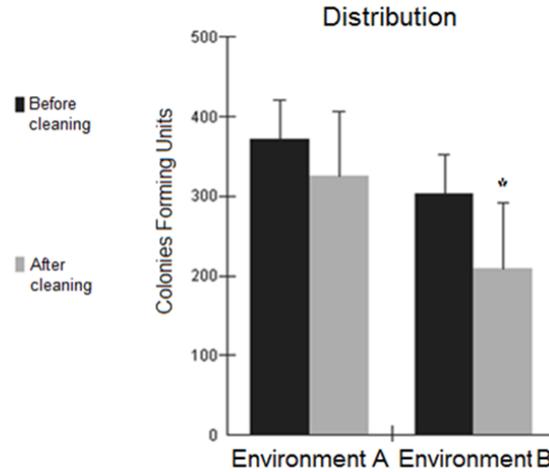
Whereas the Neonates in intensive care units are classified as high-risk patients (Cristina *et al.*, 2009), fungi is widely variable in its incidence; according to the season, temperature, relative humidity, time of day, speed and wind direction, presence of human activity and environment climate (Nevalainen *et al.*, 2015). Rangaswamy *et al.*, (2013) also reported considerable difference in the number of fungi in the morning and in the evening. Studies by Lugauskas and Kriskstaporis (2004) in hospitals and medical institutions of Lithuania showed the species *Cladosporium cladosporioides* as most frequent isolate, a similar result found in the present study.

**Table.1** Airborne fungi isolated from the two environments (A and B) before and after cleaning in the University Hospital Prof. Alberto Antunes HUPAA/Maceio, Alagoas, Brazil

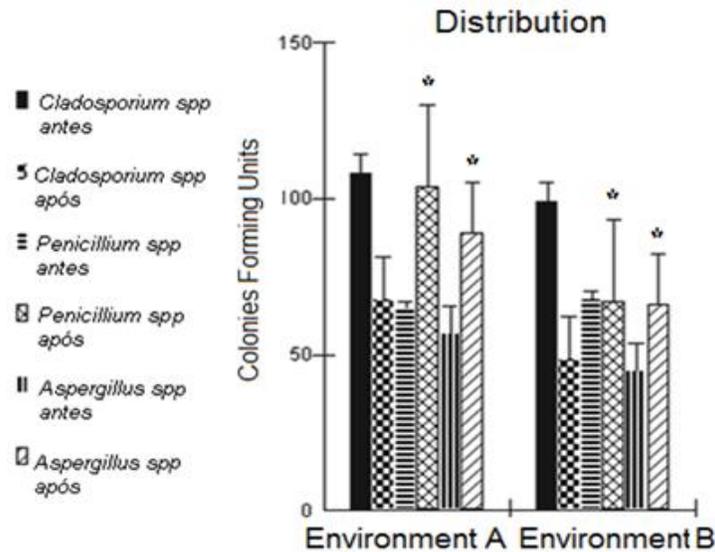
SPECIES	NEONATAL ICU ENVIRONMENT A				NEONATAL ICU ENVIRONMENT B				TOTAL
	Before cleaning		After cleaning		Before cleaning		After Cleaning		
	UFC	%	UFC	%	UFC	%	UFC	%	
<i>Aspergillus fumigatus</i> <sup>P; A</sup>	8	19,5	15	36,6	8	19,5	10	24,4	41
<i>A. nidulans</i> <sup>P</sup>	7	29,2	10	41,7	4	16,6	3	12,5	24
<i>A. niger</i> <sup>P</sup>	11	18,3	19	31,6	12	20,1	18	30,0	60
<i>A. ustus</i> <sup>P</sup>	5	26,3	7	36,8	5	26,4	2	10,5	19
<i>A. oryzae</i> <sup>P; A</sup>	26	23,0	38	33,6	16	14,2	33	29,2	113
<i>Acremonium kiliense</i> <sup>P</sup>	5	100	-	-	-	-	-	-	5
<i>Alternaria alternata</i> <sup>P</sup>	8	80	2	20	-	-	-	-	10
<i>Aureobasidium pullulans</i> <sup>P</sup>	11	22,0	9	18,0	22	44,0	8	16,0	50
<i>Bipolaris spicifera</i> <sup>P</sup>	2	100	-	-	-	-	-	-	2
<i>B. hawaiiensis</i> <sup>P</sup>	2	66,7	1	33,3	-	-	-	-	3
<i>Candida albicans</i> <sup>P</sup>	4	57,1	1	14,3	2	28,6	-	-	7
<i>C. guilliermondii</i> <sup>P</sup>	5	45,4	2	18,2	3	27,3	1	9,1	11
<i>C. parapsilosis</i> <sup>P</sup>	12	34,3	8	22,8	10	28,6	5	14,3	35
<i>C. tropicalis</i> <sup>P</sup>	3	100	-	-	-	-	-	-	3
<i>Chaetomium globosum</i> <sup>P; A</sup>	4	57,1	-	-	3	42,9	-	-	7
<i>Cladosporium cladosporioides</i> <sup>NP</sup>	64	34,4	52	27,9	48	25,8	22	11,8	186
<i>C. herbarum</i> <sup>P</sup>	18	56,2	5	15,6	9	28,1	-	-	32
<i>C. oxysporum</i> <sup>P</sup>	2	16,7	-	-	6	50	4	33,3	12
<i>C. sphaerospermum</i> <sup>P</sup>	24	25,5	11	11,7	36	38,3	23	24,4	94
<i>Curvularia giniculata</i> <sup>P</sup>	-	-	-	-	5	62,5	3	37,5	8
<i>C. lunata</i> <sup>P</sup>	4	66,7	2	33,3	-	-	-	-	6
<i>C. clavata</i> <sup>P</sup>	5	41,7	3	25,0	4	33,3	-	-	12
<i>Fusarium incarnatum</i> <sup>P</sup>	3	75,0	-	-	1	25,0	-	-	4
<i>F. subglutinans</i> <sup>P</sup>	2	66,7	1	33,3	-	-	-	-	3
<i>Geotrichum candidum</i> <sup>P; T</sup>	19	57,5	9	27,2	4	12,2	1	3,1	33
<i>Gliocladium roseum</i> <sup>NP</sup>	6	66,7	2	22,3	1	11,0	-	-	9
<i>Hortaea werneckii</i> <sup>P</sup>	1	100	-	-	-	-	-	-	1
<i>Monilia sitophila</i> <sup>NP</sup>	16	30,2	12	22,6	17	32,1	8	15,1	53
<i>Mycelia sterilia</i> <sup>NP</sup>	23	40,3	15	26,3	14	24,5	5	8,9	57
<i>Nigrospora sphaerica</i> <sup>NP</sup>	-	-	-	-	1	100	-	-	1
<i>Penicillium aurantiogriseum</i> <sup>NP</sup>	38	28,8	56	42,4	17	12,9	21	15,9	132
<i>P. chysogenum</i> <sup>P</sup>	7	19,5	12	33,3	7	19,4	10	27,8	36
<i>P. citrinum</i> <sup>P</sup>	1	8,3	5	41,7	2	16,6	4	33,4	12
<i>P. decubens</i> <sup>P</sup>	-	-	-	-	5	62,5	3	37,5	8
<i>P. expansum</i> <sup>P</sup>	10	14,7	16	23,5	28	41,2	14	20,6	68
<i>P. purpurogenum</i> <sup>P; A</sup>	4	25	6	37,5	4	25	6	37,5	16
<i>P. verruculosum</i> <sup>NP</sup>	5	17,8	9	32,1	5	17,8	9	32,1	28
<i>Rhinochadiella aquaspersa</i> <sup>P</sup>	3	37,5	1	12,5	4	50,0	-	-	8
<i>Rhizopus ozygosporus</i> <sup>P</sup>	5	41,7	4	33,4	2	16,6	1	8,3	12
<i>Rhodotorula minuta</i> <sup>P</sup>	3	75,0	-	-	1	25,0	-	-	4
<i>R. mucilaginoso</i> <sup>P</sup>	4	66,7	-	-	2	33,3	-	-	6
<i>Stachybotrys chartarum</i> <sup>P</sup>	4	80,0	-	-	1	20,0	-	-	5
<i>Syctalidium lignicola</i> <sup>P</sup>	2	100	-	-	-	-	-	-	2
<i>Syncyphalastrum racemosum</i> <sup>NP</sup>	3	37,5	1	12,5	4	50,0	-	-	8
<i>Verticillium chlamyosporium</i> <sup>NP</sup>	7	46,7	2	13,2	5	33,4	1	6,7	15
<b>TOTAL</b>	<b>400</b>	<b>30,6</b>	<b>372</b>	<b>28,5</b>	<b>318</b>	<b>24,4</b>	<b>215</b>	<b>16,5</b>	<b>1305</b>

P- pathogenic, NP- not pathogenic, T- toxigenic, A- allergenic

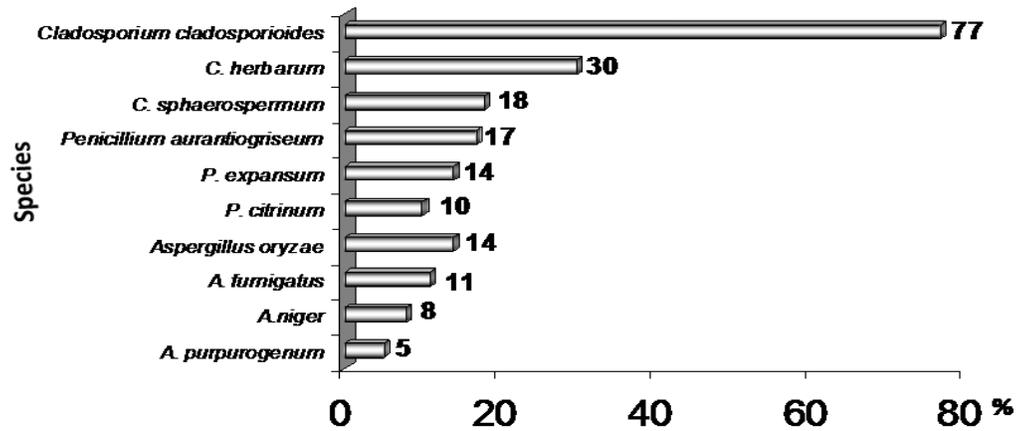
**Fig.1** Distribution of colony forming units (CFU) before and after cleaning of environments A and B at the Neonatal intensive care unit (ICU) in the hospital Dr. Alberto Antunes (Maceió – Brazil)



**Fig.2** Distribution of colony forming units (CFU) of *Cladosporium*, *Penicillium* and *Aspergillus* species before and after cleaning of environments A and B at the neonatal intensive care unit (ICU) in the hospital Dr. Alberto Antunes (Maceió – Brazil)



**Fig.3** Fungi species isolated from the air conditioner filters of two environments from the neonatal ICU in the hospital Dr. Alberto Antunes (Maceió – Brazil)



In Brazil, there are few data on the airborne fungi in hospital environments, especially those related to identification to species level.

The species *C. cladosporioides* was also found in 80% of isolates from air conditioners filters. However, Morbim and Salmito (2006) when evaluating the mycoflora from air conditioners in the ICU of Teresina - Brazil observed that *Aspergillus niger* was the most commonly species found in 60% of equipment, followed by *Aspergillus fumigatus* and *Trichoderma koningii* also present in this study in less frequency, with the exception of *Trichoderma koningii* that was not found in this study. The air conditioners in window, split or central concentrates microorganisms in the filters, evaporating unit and stagnant water from the pan. This fact associated with the cumulative phenomenon of 90% recycled air promotes increase in the number of microorganisms in order 1.000-100.000 times compared to the external environment (Lacerda, 2000). The air recirculation and the microorganisms are a hazard to individuals who remain for a long time inhaling the air, this may cause respiratory tract infections such as sinusitis, rhinitis, tonsillitis, pharyngitis, bronchitis, pneumonia, asthma, colds and flu. In this study, we report the presence of pathogenic, toxigenic and allergenic fungi in the air of two

neonatal intensive care units. For one of the evaluated ICUs, the cleaning procedure was not sufficient to reduce airborne fungal concentration, which may be important when considering the immunological condition of patients present in this environment.

Among the filamentous fungi identified, *C. cladosporioides*, *P. aurantiogriseum* and *A. oryzae* were the most prevalent species in the analyzed environments. Regarding yeast fungi, *Candida* species such as *C. parapsilosis*, *C. guilliermondii*, *C. tropicalis* and *C. albicans* were present, demonstrating that indoor air may be an important risk factor for the spread of infection-related pathogens in neonates.

Completely eliminate the risk of health problems caused by fungi is practically impossible, but it can be reduced to a minimum standard level. Regular monitoring is essential for air control quality and for detecting the presence of hazardous microorganisms to human health and these data can be used to define specific indoor air quality guidelines at hospital environments.

The present study shows new results about fungi species found in the hospital environments of neonatal ICU in Brazil,

providing a valuable tool for pathogens control and decrease in the level of infection in air-conditioning hospital environments.

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#### **How to cite this article:**

Aryanna Kelly Pinheiro Souza, Jean Phellipe Marques do Nascimento, Maria Anilda dos Santos Araújo, Krystianelly Patrícia da Silva Pedrosa, Bruno Mendes Tenorio, Luana Luzia Santos Pires, Gabryelle Barbosa Cordeiro de Lima, Rebeca Ingrid dos Santos Barboza and Eurípedes Alves da Silva Filho. 2019. Airborne Fungi in Neonatal Intensive Care Unit of a Public Hospital in Brazil. *Int.J.Curr.Microbiol.App.Sci*. 8(12): 1210-1219. doi: <https://doi.org/10.20546/ijcmas.2019.812.149>