Airborne Fungi in a Neonatal Intensive Care Unit and Operating Theater in a University Hospital

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ABSTRACT

Monitoring the microbiological quality of indoor air in hospital environments is a matter of comprehensive discussion due to its influence on the transmission and spread of pathogenic microorganisms. Among the artificially air-conditioned environments, hospitals are noteworthy for being specific places for the treatment and recovery of patients. In addition to problems related to patients health and professionals health, immunocompromised patients are more exposed to microorganisms present in the air currents of the refrigeration system in these environments, which can lead to consequences such as the occurrence of outbreaks. The objective of this work was to evaluate the indoor air quality in critical hospital environments of a teaching hospital in the city of Maceió, the state of Alagoas. In addition, we sought to identify the anemophilous fungal microbiota present. Air collections were taken in the rainy season, totaling, following recommendations indicated by Resolution No. 9 of the Brazilian National Health Surveillance Agency. The study was based on determining the concentration parameter of bioaerosols in indoor and outdoor air. The fungal microbiota identification was carried out by analyzing macro and microscopic characteristics for filamentous fungi and the use of molecular tools for yeasts. The most frequent species in hospital critical environments were Cladosporium cladosporioides, Penicillium piceum, Penicillium aurantiogriseum, Cladosporium herbarum and Aspergillus oryzae. In outdoor air, the most frequently found fungi were Penicillium sp., Aspergillus sp., and Cladosporium species. Candida tropicalis, C. krusei, and C. parapsilosis were identified among the yeasts in indoor and outdoor air samples. Identifying potentially pathogenic fungi in the evaluated environments points to the need for continuous monitoring of indoor air quality. Furthermore, to avoid the widespread fungal pathogens and the consequent occurrence of outbreaks, the adoption of indoor air microbiological quality analysis programs is suggested as an essential tool in developing infection control standards. In our study, airborne fungi are

reported as indoor air contaminants in critical hospital environments. This finding is noteworthy because, in general, individuals present in these environments have an immunological impairment.

Keywords: airborne fungi, indoor air quality, yeasts, fungal infection, indoor environments.

Introduction

The impact of indoor air quality on the health and well-being of individuals in built environments has been the subject of numerous studies in the field of public health (VAN TRAN et al. 2020). The most critical consequence of poor air quality is undoubtedly related to human health, especially with the spread of important pathogens such as fungi and yeasts (SATTAR, 2016). Several studies have reported the presence of fungal bioaerosols in indoor air in environments such as schools, offices, and homes (ABDEL-AZIZ and RADWAN, 2020; GUO et al. 2020).

In hospital environments, poor indoor air quality can be even more worrisome due to individuals in critical areas who often present some immunological impairment. In this specific environment, the occurrence of a great diversity of fungi can favor the emergence of infections, specifically by opportunistic species (DO NASCIMENTO et al. 2019). Souza et al. (2019) evaluated indoor air contamination in neonatal intensive care units (neonatal ICU) and found tremendous fungal diversity in their analyses. Important fungal pathogens such as *Aspergillus fumigatus*, *Cladosporium cladosporioides*, and *Candida* spp. have been detected. Thus, in addition to diseases dissemination, air quality can directly influence the speed of patients' recovery and length of hospital stay. This aspect must be considered, as studies have shown that a long period of hospital stay generates various types of losses. Furthermore, generate increased hospital costs, high rates of admission to intensive care units (ICU), and acquisition of serious infections (HOOGERVORST -SCHILP et al. 2015).

The occurrence of fungal infections in the hospital context has increased significantly in recent years. Poor indoor air quality has played a highly relevant role (STOCKWELL et al. 2019). The literature has frequently reported the relationship between airborne contaminants and fungal disease outbreaks (SAAD-HUSSEIN and IBRAHIM, 2021; DEY et al. 2019). Given the occurrence and pathogenic potential of many opportunistic airborne fungi, monitoring indoor air quality and knowledge of the fungal species present in these locations can provide vital information for developing infection control strategies. Therefore, this study aimed to evaluate the indoor air quality in critical environments of a university hospital in the city of Maceió. Furthermore, we sought to investigate the fungal diversity present in the collected samples, mainly checking the occurrence of possibly pathogenic species.

Material and methods

Sample collection

The study was carried out in critical hospital areas where patients with compromised or weakened health status are frequently observed. Three neonatal intensive care units (Neonatal ICU) and six operating room in a teaching hospital in the city of Maceió, State of Alagoas, were selected. Air collections were taken in a rainy season (March - September).

Collections in hospital environments were carried out according to the recommendations of Resolution No. 09 (RE No. 09) of the National Health Surveillance Agency – ANVISA (BRASIL, 2003). This document establishes reference standards for indoor air quality in artificially air-conditioned environments for public and collective use. In addition to air-conditioned indoor air collections, we collected atmospheric air to verify fungal diversity outside the hospital environment.

For the investigation of fungal bioaerosols, a one-stage Anderson® impaction air sampler was used. The equipment was positioned in the center of environments analyzed at the height of 1.5 m from the ground. At each sampling point, an airflow rate kept fixed at 28.3 L/min for 10 minutes was used, totaling a volume of air collected per sample of 283 liters. Disposable Petri dishes (90 x 10 mm) containing the Agar Sabouraud Dextrose medium with chloramphenicol (0.5 g/L) for bioaerosol collections were used. The equipment was disinfected with gauze soaked in 98% isopropyl alcohol during the collection interval to avoid cross-contamination.

Quantitative and qualitative analysis of colonies

After each collection, the plates were identified, placed in plastic bags, and transported to the laboratory. Fungal growth was observed from incubation of collection plates in an oven at 25 ºC ± 1ºC for seven days. Colonies were quantified in colony-forming units per cubic meter of air (CFU/m3).

In addition to quantification, filamentous fungi were isolated and identified based on macroscopic and microscopic characteristics (LACAZ et al. 2002; RIDDELL, 1950). Filamentous fungi identification was based on macroscopic aspects of colonies and microscopic characteristics by directly examining the culture, according to Hoog et al. (2000). Those which the absence of reproductive structures could not identify were grown in a specific medium to stimulate sporulation using the in-slides microculture technique, according to Riddell (1950). The isolates were stored in sterilized distilled water and preserved using the Castellani method (CASTELLANI, 1963).

Molecular identification of yeasts

Yeast isolates were identified by the polymerase chain reaction (PCR) technique. Initially, pure yeast cultures were inoculated individually in 1.0 mL of liquid YPD (Yeast Extract Peptone Dextrose) medium and incubated at 30 °C for 16 hours at 150 rpm on a shaking table at controlled temperature. After this period, the contents were transferred to sterilized 1.5 mL microtubes and centrifuged at 5000 g for three minutes. The supernatant was discarded, and the pellet was washed in 1.0 mL of sterile distilled water and again centrifuged at 5000 g for three minutes. After this step, the samples were ready for DNA extraction.

DNA extraction was performed by adding 600 µL of lysis solution to the cell pellet (2.0 mL 1 M Tris-HCl pH 8.0, 0.5 mL 0.5 M EDTA pH 8.0, 1.0 mL 10% SDS, 0.5 mL 0.5 M NaCl and 6.0 mL sterile distilled water). The microtubes were kept at 65 °C in a water bath for 30 minutes with inversion shaking every 10 minutes. Subsequently, an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added, and after brief vortexing, the suspension was centrifuged at 12,000 g for 10 minutes. Then, 500 µL of the aqueous phase was transferred to new sterilized 1.5 mL microtubes, and 500 µL of chloroform/isoamyl alcohol (24:1) was added. The samples were centrifuged again for the same period and

rotation. After this procedure, 400 µL of the upper phase was transferred to new sterilized microtubes, and 800 µL of absolute cold ethanol was added, remaining for 2 hours at -20 °C for DNA precipitation. Then, the microtubes were kept at room temperature for 20 minutes and centrifuged at 12,000 g for 10 minutes. Precipitated DNA was washed in 70% ethanol twice and dried at room temperature (± 25 °C). DNA was resuspended in Tris/EDTA (TE) buffer pH 8.0 (10 mM TRIS/1 mM EDTA) and stored at -20°C for further amplification.

PCR reactions were performed using species-specific yeast primers, as described in the literature (Table 1). Reactions were performed in a final volume of 20 µL in a Peltier Thermal Cycler MJ25+ (MJ Research/Bio-Rad). Amplification cycles were: initial denaturation of 5 minutes at 96 °C, followed by 40 cycles with denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds, extension at 72 °C for 30 seconds, and one step of final extension at 72°C for 15 minutes. The amplified products were separated by 1.3% agarose gel electrophoresis. After electrophoresis, we stained the gel in ethidium bromide for two minutes. The visualization was made in a transilluminator and photographed in a documentation gel system (Doc-Print II - Vilber Lourmat). DNA from yeasts from the Micoteca Culture Collection at the Federal University of Pernambuco – URM/UFPE was used as a positive control. The isolates obtained were: *Candida albicans* URM5689; *C. parapsilosis* URM5583; *C. guilliermondii* URM5563; *C. glabrata* URM5594; *C. tropicalis* URM5694 and *C. krusei* URM1059.

Table 1 - Species-specific primers for the identification of pathogenic yeasts of the genus *Candida*.

Species	Primers	Sequence (5' – 3')	bp	References
Candida albicans	CALB1	TTT ATC AAC TTG TCA CAC CAG A	≈273	**Luo and Mitchell, 2002**
	CALB2	ATC CCG CCT TAC CAC TAC CG		
Candida glabrata	CGL1	TTA TCA CAC GAC TCG ACA CT	≈423	**Luo and Mitchell, 2002**
	CGL2	CCC ACA TAC TGA TAT GGC CTA CAA		
Candida parapsilosis	CPA1	GCC AGA GAT TAA ACT AAC CA	≈300	**Hsu et al. 2003**
	CPA2	CCT ATC CAT TAG TTT ATA CTC CGC		
Candida tropicalis	CTR1	CAA TCC TAC CGC CAG AGG TTA T	≈357	**Luo and Mitchell, 2002**
	CTR2	TGG CCA CTA GCA AAA TAA GCG T		
Candida guilliermondii	CGU1	GCA TCG ATG AAG AAC GCA GC	≈315	**Hsu et al. 2003**
	CGU2	GTT TGG TTG TTG TAA GGC CGG G		
Candida kruzei	**CKRU1**	**GCA TCG ATG AAG AAC GCA GC**	**≈258**	**Hsu et al. 2003**
	CKRU2	**AAA AGT CTA GTT CGC TCG GGC C**		

Statistical analysis

We performed a descriptive statistical analysis of the data obtained and the determination of fungal genus frequency and species identified in our investigation. All analyzes were performed using GraphPad Prism version 8.3.0 software.

Results

In this study, 12 indoor air collections were performed in critical hospital environments such as Neonatal Intensive Care Units (NICU A, NICU B, NICU C) and Surgical centers. In addition, we also carried out 12 outdoor air collections to verify fungal diversity outside hospital areas. We found a considerable variation in fungal concentration between the hospital environments analyzed, with a minimum and maximum value of 11 and 600 CFU/m³, respectively, and a mean value of 131.6 CFU/m³ ± 131.8. Values referring to the minimum, maximum and average concentrations for each evaluated hospital environment and the values of outdoor air samples are available in table 2.

Table 2 – Airborne fungal concentration (Colony Forming Unit -CFU) in indoor hospital critical areas and outdoor air

	NICU A	NICU B	NICU C	SURGICAL CENTER	OUTDOOR AIR
Minimum	32	11	17	18	39
Median	77,5	125,5	107,5	139,5	123,5
Maximum	247	565	600	300	368
Mean	87,25	158,8	148,6	139,8	151,2
Std. Deviation	59,94	152,5	158,7	81,77	110,8

Among the samples from critical hospital environments (NICU A, NICU B, NICU C, and Surgical Center), the most frequent genera were *Penicillium* with 176 (31.26%) isolated, followed by *Cladosporium* with 132 (23.45%), the group Mycelia sterilia with 97 (17.23%) and the genus *Aspergillus* with 75 (13.32%). The genera with lower frequency among samples presented a percentage < 1%. Regarding the outdoor air samples, the genus *Penicillium* was the most frequent with 44 (30.10%) isolated, followed by Mycelia sterilia group with 25 (16.69%) and the genus *Aspergillus* with 23 (13.69%) and *Cladosporium* with 20 (11.90%). The genera with lower frequency among the samples presented a percentage < 1%. The frequency distributions of genres in both indoor and outdoor air are shown in figures 1 and 2.

Figure 3 shows the general distribution of the most frequent fungal species in analyzed indoor hospital critical environments. The most frequent species were *Cladosporium cladosporioides*, *Penicillium piceum*, *Penicillium aurantiogriseum*, *Cladosporium herbarum* and *Aspergillus oryzae*. Regarding the distribution of species by a specific environment, NICU A had *C. cladosporioides*, *P. aurantiogriseum*, and *C. sphaerospermum* as the most frequent species; NICU B had *C. cladosporioides*, *Aspergillus niveus*, *C. herbarum*, *A. oryzae*, and *P. piceum* as the most frequent; NICU C had *P. aurantiogriseum*, *A. oryzae* and *C. cladosporioides* as the most frequent. Regarding surgical centers, *P. piceum* and *C. cladosporioides* were the most frequent.

Figure 1 – Frequency of airborne fungal genus in indoor air samples in Neonatal Intensive Care Unit (NICU) and Surgical Centers.

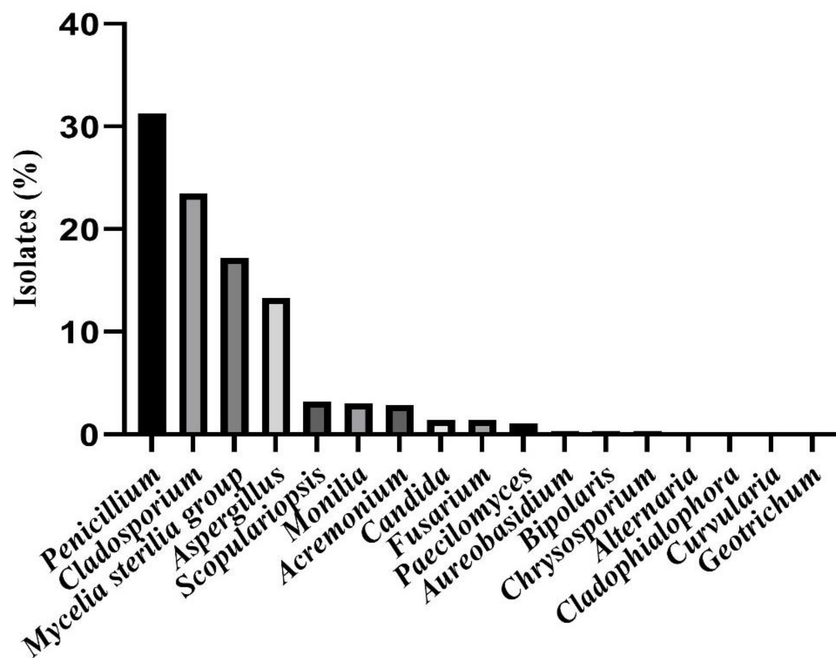


Figure 2 - Frequency of airborne fungal genus in outdoor air samples.

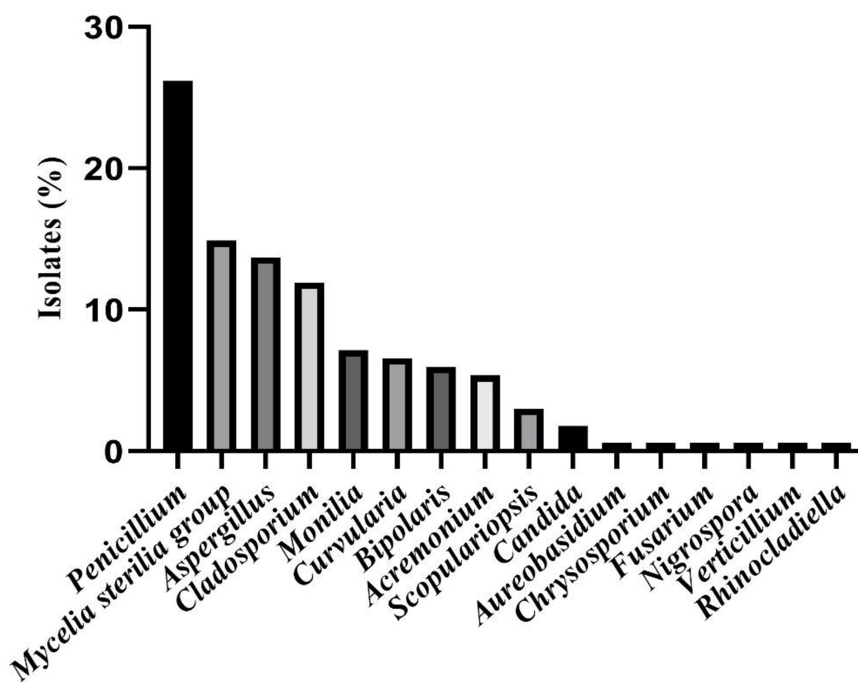
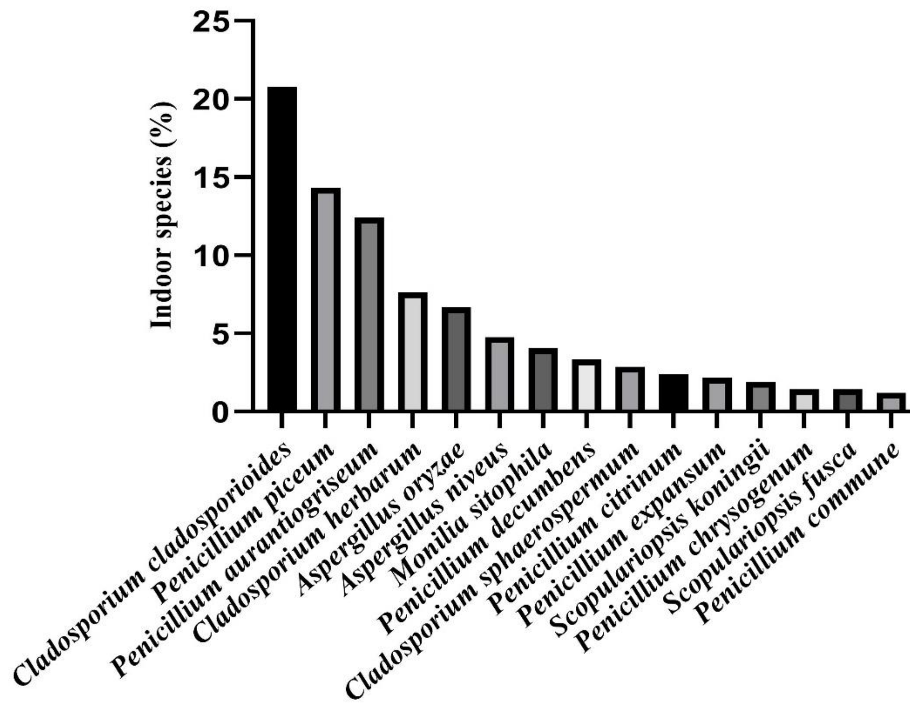


Figure 3 - Distribution of airborne fungal species identified in indoor hospital critical areas. Species with a percentage of < 1% were omitted from the graph.



We observed that some fungi occurred only in indoor air samples from critical hospital areas. The identified species were: *Aspergillus candidus*, *Aspergillus deflectus*, *Aspergillus granulosus*, *A. niveus*, *Aspergillus sclerotiorum*, *Aspergillus ungüis*, *Acremonium hyalinulum*, *Alternaria alternata*, *Aureobasidium pullulans*, *Bipolaris hawaiiensis*, *Cladosporium oxysporium*, *C. sphaerospermum*, *Cladophialophora bantiana*, *Fusarium solani*, *Geotrichum candidum*, *Paecilomyces variotii*, *Penicillium brevicompactum*, *Penicillium commune*, *Penicillium griseofulvum*, *Penicillium rugulosum*, *Penicillium spinulosum* e *Scopulariopsis fusca*. Concerning outdoor air, we also verified the specific occurrence of some species. The species *Aspergillus fumigatus*, *Bipolaris australiensis*, *Curvularia brachyspora*, *Nigrospora sphaerica* and *Scopulariopsis brumptii* were only present in outdoor air samples. Table 3 shows the distribution of all fungi identified in this study and their occurrence in each environment and sample analyzed.

Table 3 - Distribution and identification of airborne fungi species in air samples of the hospital critical areas and outdoor air.

Species	NICU A	NICU B	NICU C	Surgical Center	Outdoor air	Total
Aspergillus candidus [P]				3 (2.88%)		3
Aspergillus deflectus [P]				1 (0.96%)		1
Aspergillus fumigatus [P; A]					7 (6.25%)	7
Aspergillus granulosus [P]		1 (0.74%)				1
Aspergillus nidulans [P]		1 (0.74%)			1 (0.89 %)	2
Aspergillus niger [P]				1 (0.96%)	1 (0.89 %)	2
Aspergillus niveus [P]		20 (14.81%)				20
Aspergillus ochraceus [P]		1 (0.74%)	1 (1.54%)		1 (0.89%)	3
Aspergillus oryzae [P; A]	3 (2.61%)	15 (11.11%)	8 (12.31%)	2 (1.92%)	10 (8.93%)	38
Aspergillus sclerotiorum [P]			1 (1.54%)			1
Aspergillus ungüis [P]	2 (1.74%)					2
Acremonium hyalinulum [P]	1 (0.87%)	1 (0.74%)				2
Acremonium kiliense [P]	1 (0.87%)	1 (0.74%)			1 (0.89%)	3
Acremonium patronii [P]				2 (1.92%)		2
Acremonium recifei [P]	1 (0.87%)	2 (1.48%)		1 (0.96%)		4
Alternaria alternata [P]	1 (0.87%)					1
Aureobasidium pullulans [P]			1 (1.54%)	1 (0.96%)		2
Bipolaris australiensis [P]					10 (8.93)	10
Bipolaris hawaiiensis [P]	1 (0.87%)	1 (0.74%)				2
Candida krusei [P]		1 (0.74%)		1 (0.96%)	1 (0.89%)	3
Candida parapsilosis [P]		1 (0.74%)	1 (1.54%)	1 (0.96%)	1 (0.89%)	4
Candida tropicalis [P]	1 (0.87%)		1 (1.54%)	1 (0.96%)	1 (0.89%)	4
Cladosporium cladosporioides [NP]	33 (28.70%)	22 (16.30%)	8 (12.31%)	24 (23.08%)	15 (13.39%)	102
Cladosporium herbarum [P; A]	3 (2.61%)	18 (13.33%)	3 (4.62%)	8 (7.69%)	4 (3.57%)	36
Cladosporium oxysporium [P]	1 (0.87%)					1
Cladosporium sphaerospermum [P]	11 (9.57%)			1 (0.96%)		12
Cladophialofora bantiana [P]			1 (1.54%)			1
Curvularia brachyspora [P]					7 (6.25%)	7
Curvularia clavata [P]		1 (0.74%)			4 (3.57%)	5
Fusarium solani [P]		4 (2.96%)				4
Geotrichum candidum [P; T]	1 (0.87%)					1
Monilia sitophila [NP]	1 (0.87%)	7 (5.19%)	5 (7.69%)	4 (3.85%)	3 (2.68%)	20
Nigrospora sphaerica [P]					1 (0.89%)	1
Paecilomyces variotii [P; A]	1 (0.87%)					1
Penicillium aurantiogriseum [NP]	27 (23.48%)	6 (4.44%)	16 (24.62)	3 (2.88%)	11 (9.82%)	63
Penicillium brevicompactum [P]		1 (0.74%)				1
Penicillium citrinum [P]		1 (0.74%)	2 (3.08%)	7 (6.73%)	1 (0.89%)	11

P: Pathogenic; T: Toxigenic; A: Allergenic; NP: Nonpathogenic (De Hoog *et* al. 2000).

Continues

Table 3 - Distribution and identification of airborne fungi in air samples of the hospital critical areas and outdoor air.

Species	NICU A	NICU B	NICU C	Surgical Center	Outdoor air	Total
Penicillium commune P		1 (0.74%)		4 (3.85%)		5
Penicillium chrysogenum P	4 (3.48%)		2 (3.08%)		2 (1.79%)	8
Penicillium decumbens P	4 (3.48%)	7 (5.19%)	2 (3.08%)	1 (0.96%)	4 (3.57%)	18
Penicillium expansum P	2 (1.74%)	1 (0.74%)	3 (4.62%)	3 (2.88%)	4 (3.57%)	13
Penicillium griseofulvum NP				1 (0.96%)		1
Penicillium piceum P	7 (6.09%)	13 (9.63%)	6 (9.23%)	34 (32.69%)	17 (15.18%)	77
Penicillium rugulosum P	1 (0.87%)					1
Penicillium spinulosum P		2 (1.48%)				2
Scopulariopsis brevicaulis P	1 (0.87%)	3 (2.22%)			1 (0.89%)	5
Scopulariopsis brumptii P					1 (0.89%)	1
Scopulariopsis fusca P	1 (0.87%)	1 (0.74%)	4 (6.15%)			6
Scopulariopsis koningii P	6 (5.22%)	2 (1.48)			3 (2.68%)	11
Total	115 (100%)	135 (100%)	65 (100%)	104 (100%)	112 (100%)	531

P: Pathogenic; T: Toxigenic; A: Allergenic; NP: Nonpathogenic (De Hoog *et* al. 2000).

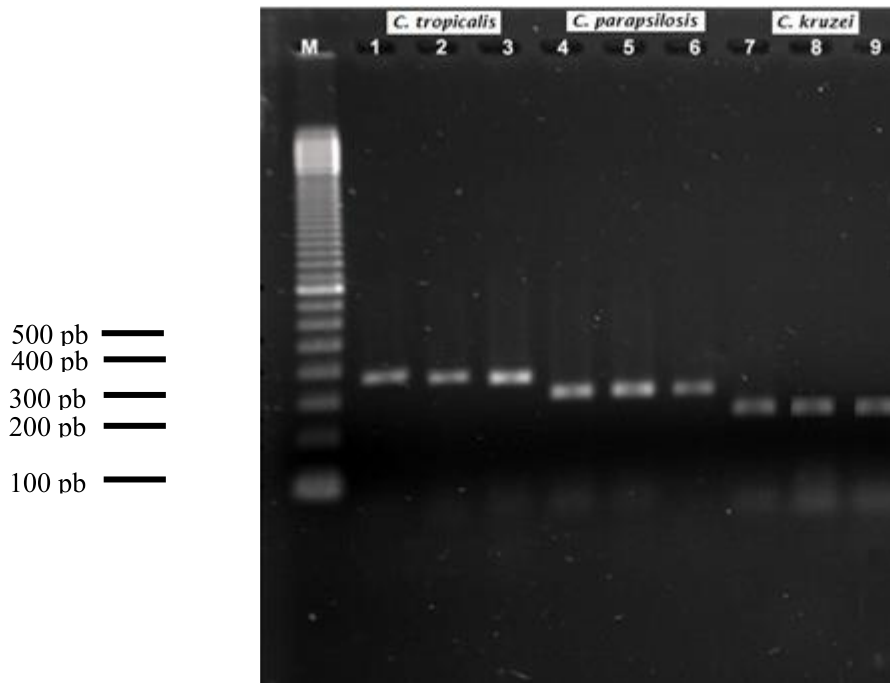
We obtained 85 yeast isolates from the collected samples, with 19 (22.35%) isolated from NICU A, 12 (14.12%) from NICU B, 22 (25.88%) from NICU C, and 19 (22, 35%) from the surgical center. From outdoor air samples, we obtained 13 (15.30%) isolates. Out of the total isolated yeasts (n=85), 71 (83.52%) were contaminated with spores of filamentous fungi, 11 were selected for molecular identification, and three (3.53%) could not be identified by the approach used in this study.

Of the 11 isolates identified by PCR with species-specific primers, there was amplification with positive isolates for *Candida tropicalis*, *C. krusei*, and *C. parapsilosis* species (Figure 3). All species were present in both indoor and outdoor air samples.

Discussion

In this survey, we verified a great aerial fungal diversity in critical hospital areas. Of great relevance, individuals present in these environments are generally in conditions that can favor the occurrence of opportunistic infections, especially in those who present some immunological impairment. Thus, air quality monitoring is an effective tool to avoid the possibility of fungal infections outbreaks via indoor air. In addition, several studies have highlighted the relevance of indoor air quality in hospital environments and the risks associated with the occurrence of airborne fungi, especially in critical hospital environments. (Belizario et al. 2021; Do Nascimento et al. 2019).

Figure 3 - Electrophoresis gel showing species-specific identification of *Candida*. Lines from M to 9; M - 100 base pair (bp) molecular weight marker; 1 - *C. tropicalis* URM 5694 (357 bp); 2 – AY17*; 3 – AY18*; 4 - *C. parapsilosis* URM 5583 (300 bp); 5 – AY1*; 6 – AY5*; 7 - *C. krusei* URM 1059 (258 bp); 8 – AY2*; 9 – AY6*.



*AY – Airborne Yeast.

In our results found fungal concentrations ranging from 11 to 600 CFU/m³, with an average value of 131.6 CFU/m³. Comparing these findings with other studies, we found significant differences in fungal concentrations reported in critical hospital environments. Nasiri et al. (2021) investigated the microbiological air quality in different hospital wards (NICU and Surgical center) and found that the mean concentrations obtained for each environment were much lower than those found by us. Likewise, Mirhoseini et al. (2020) and Montazeri et al. (2020) reported fungal concentrations in critical hospital areas that ranged from 0 to 63 CFU/m³ and 7 to 92 CFU/m³ respectively, and mean concentrations that were also below that found in this study. This difference in the results reported by us compared to the studies mentioned above can be attributed to factors of the evaluated environments, influencing the increase in fungal concentrations. Considering the particularity of environments evaluated related to the microbial contamination minimize, the HVAC system may have contributed to the high fungal concentrations observed. Evidence suggests that poorly maintained HVAC systems may favor the multiplication and spread of pathogenic microorganisms via indoor air (SANTOS et al. 2020; BOŽIĆ et al. 2019). We noted that these environments did not have a central air system, and cooling was done by SPLIT-type devices that do not promote the renewal of ambient air. Thus, the lack or deficiency in air renewal in analyzed environments may have contributed decisively to the increase in airborne fungi concentration.

We found a considerable diversity of fungal genera present in the indoor air of critical hospital environments. *Penicillium* (31.26%), *Cladosporium* (23.45), Mycelia sterilia group (17.23%), and *Aspergillus* (13.32%) were the most frequent in our samples, and this corroborates the results of other

studies. Souza et al. (2019) evaluated fungal air contamination in two NICUs and reported *Cladosporium*, *Penicillium*, and *Aspergillus* as the most frequent. Like us, Gonçalves et al. (2018) found *Penicillium* as the most frequent in evaluations of fungal contamination in an intensive care unit (ICU), followed by *Aspergillus* and *Cladosporium*. Jalili et al. (2021) investigated airborne bacterial and fungal concentration in different hospital wards and verified that yeasts, *Penicillium*, and *Aspergillus* were the only fungi found. The low fungal diversity reported by these authors compared to our results suggests that the optimal functioning of the hospital HVAC system and the absence of factors that facilitate fungal growth in the environment may be determinants to minimize the risk of exposure to airborne pathogens. Thus, air quality monitoring studies provide data that can be important for understanding the factors involved in the aerial pathogen's dissemination. In addition, it helps to develop effective strategies in reducing fungal growth within the hospital environment.

In many studies on monitoring the microbiological quality of indoor air, species of the genera *Penicillium*, *Aspergillus*, and *Cladosporium* make up the primary fungal contaminants of indoor air in critical and non-critical hospital environments. Our results are in line with this finding and corroborate the reports by Souza et al. (2019) and Demirel et al. (2017), who also found tremendous fungal diversity in NICU and identified members of these groups more frequently. Although these fungi are the most found in indoor hospital air quality analyses, our findings indicate that species of other genera may also be important in the composition of aeromycoflora in indoor hospital environments. Like us, other studies have reported other groups of airborne fungi composing the indoor mycobiota of critical and non-critical hospital environments (DHAR et al. 2021; LARREY et al. 2020; KIASAT et al. 2017).

Considering the uniqueness of the environments studied in our monitoring and the condition of individuals present in these locations, our findings are noteworthy concerning the possibility of exposure to opportunistic human pathogens. Similarly, mycotoxins and fungal allergens can also pose an additional hazard when exposed to poor indoor air. In our monitoring, we observed the presence of a great diversity of fungal species in indoor air samples in critical hospital environments. As shown in table 3, many of these fungi are considered human pathogens and producers allergenic, toxigenic, and volatile organic compounds substances that may offer an additional risk of exposure. Furthermore, our results corroborate current evidence regarding the importance of indoor air in the pathogen's spread within the hospital environment, like the report made by Sattar (2016).

In their study, Egbuta et al. (2017) describe the risks to human health associated with exposure to filament fungi and the substances they produce. Species such as *A. nidulas*, *A. niger*, *A. ochraceus*, *F. solani*, *P. citrinum*, *P. expansum*, *P. griseofulvum*, *P. rugulosum*, and *P. aurantiogriseum* are reported as mycotoxin producers. We observed these species in the hospital environments analyzed in our study. In line with us, Rasmey et al. (2018) and Heutte et al. (2017) also reported potentially mycotoxin-producing airborne fungi in the indoor air hospital environment. Of equal significance, many of the fungi found in our monitoring are opportunistic pathogens and have been reported as etiologic agents of various types of infections. In highly susceptible patients, *A. ochraceus* and *A. niger* have been associated with aspergillosis (Hakamifard et al. 2021; Atchade et al. 2017). *P. chrysogenum* and *P. citrinum* were associated with pulmonary infection in patients who underwent organ transplants (Beena et al. 2021; Geltner et al. 2013). Godoy and coauthors (2004) reported *F. solani* as the etiological agent of onychomycosis in Brazil, while

Pérez-Cantero and Guarro (2020) discuss the aspects involved in infections caused by *Scopulariopsis* sp. Based on these aspects, we reinforce the need for care related to hospital indoor air quality in critical areas, mainly to prevent the occurrence of infections that can be fatal in immunocompromised patients.

In addition to filamentous fungi, we check for the presence of yeasts in critical hospital areas. Although we only detected the presence of *C. krusei*, *C. parapsilosis*, and *C. tropicalis*, it is possible that the diversity of yeasts in the analyzed environments was greater, mainly because the approach we used restricted the identification of other species. Unlike the findings by Awosika et al. (2012), we do not detect *Candida albicans* in our indoor air samples. However, given the large number of isolates that our strategy could not identify, we cannot exclude their presence in the analyzed environments. This is because this species is often reported as an indoor air contaminant in indoor environments.

Non-albicans Candida has also been highlighted as crucial indoor air contaminants in the hospital environment. Our results corroborate those described by Calumby et al. (2019) and Cordeiro et al. (2010), who also verified the occurrence of this yeasts' group in critical hospital areas. The occurrence of non-albicans Candida in critical areas is worrying when we consider the compromised immunological condition of patients and the fact that some species are resistant to the antifungal agents currently used in therapy. In line with this, Gong et al. (2016) and Zakhem et al. (2021) reported non-albicans Candida as the causal agent of invasive candidiasis and candidemia, respectively. In their papers, the authors described *C. tropicalis* and *C. parapsilosis* as the causative agent of the infections, and we verified these yeasts in our samples. *C. krusei*, another non-albicans Candida identified in our study, has aroused concern as it can develop a profile of multidrug resistance to the main classes of currently used antifungals (Jamiu et al. 2021). Given the above, the precise identification of airborne yeast species in critical hospital areas is essential to know the risks of exposure in these environments. Thus, molecular tools such as the strategy used in our study or through the sequencing of yeast DNA barcoding can be essential in understanding the microbial diversity present in indoor air hospital.

Based on the airborne fungal concentration, diversity of the genus and species in outdoor air in our results, we can assume that there was no difference regarding the presence of indoor and outdoor airborne fungi. This corroborates the findings by Rostami et al. (2017), who also did not observe any difference between aerial fungal concentrations. Regarding genus, there was no difference between those that were more frequent indoor and outdoor, and in both samples, *Penicillium*, *Cladosporium*, and *Aspergillus* were the most frequent. These findings align with Ziaee et al. (2018), who also verified this genus as the most frequent in their samples. Regarding fungal species, few species were identified only in outdoor air. Although the hypothesis that outdoor air influences the indoor fungal concentration is accepted, at least concerning the diversity of species found, it is possible that internal factors have contributed to greater diversity in the evaluated environments. In our 11-year experience working with indoor air quality, we found that indoor air constitutes a significant risk factor in transmitting bacterial, fungal, and viral pathogens. High concentrations of microorganisms may be present in indoor air, mainly due to the lack of proper periodic maintenance of the HVAC system and the absence of air renewal. The adoption of these precautions has been observed in most emerging countries.

Conclusion

In our monitoring, a high fungal concentration in critical hospital areas was verified, and the immunosuppressive condition of individuals present in these environments deserves to be highlighted. We observed a great diversity of fungal genera in both the indoor and outdoor air samples with 92% of potentially pathogenic species. Among the genus found, *Penicillium*, *Cladosporium*, and *Aspergillus* were the most frequent in both samples. At the species level, a more significant amount was observed in indoor environments than outdoor air, which seems to have been influenced by factors within the hospital environment. Thus, studies of the microbiological indoor air quality are essential in revealing the mycobiota present in critical hospital areas and developing strategies that help minimize the risks of exposure.

Conflict of Interest

There are no conflicts of interest to declare.

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