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Assessment of the abundance and diversity of airborne fungi in two different air conditioning systems in Paraíba, Brazil

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***Abstract*— *Indoor air quality is directly related to the health of individuals, and when air conditioning systems have poor sanitation and lack of adequate monitoring, they become sources of potentially pathogenic organisms. Thus, the aim of this study was to evaluate internal contamination in two different environments located in the city of João Pessoa, state of Paraíba, Brazil, which use different forms of air conditioning, analyzing fungal quantity and abundance. The analysis of the conventional air conditioning system and the air conditioning system that uses air renewal was performed using a bio-aerosol impactor to quantify the pathogens. Later, the fungi identification of the air samples was carried out by the slide microculture technique. The conventional air conditioning system, used in the health clinic, showed a greater amount of anemophilic fungi in some sectors compared to the sectors of the judicial public sector, which use the system with air renewal. The health clinic's air samples indicated that nine of the eleven sectors analyzed had a fungal density above the acceptable limit according to the current national regulatory standard, and in judicial public sector, two of the five were above this limit. In both establishments Aspergillus niger was detected, i.e. 7% in the kitchen pantry of the judicial public sector, 2% in the operating room, and 1% in the kitchen pantry of the health clinic. The results presented in this study indicate the need for better hygiene measures for air conditioning units, as well as periodic monitoring of air quality in these environments.*

***Keywords*— Anemophiles, air quality, Aspergillus niger, Bioaerosols, Monitoring.**

I. INTRODUCTION

Indoor air quality is essential for maintaining the individual's health, especially those who stay there for a long time. Currently, most of the indoor environments are air-conditioned, in order to provide comfort to occupants. However, some aspects of these systems must be taken into account, such as the periodic need to clean the equipment used in the indoor temperate control (Dehghan et al., 2018).

The correct hygiene of air conditioning equipment aims to avoid the presence of bioaerosols, which include airborne pathogens, viruses, bacteria, and fungi (Fernstrom and Goldblatt, 2013). It is important to note that environmental factors substantially influence the proliferation of these microorganisms. These air-conditioned systems provide an ideal environment for the growth of these microorganisms, due to the high humidity rates and the accumulation of impurity in the devices (Hatayama et al. 2018). Exposure to these types of pathogens deserves attention, especially in individuals with chronic respiratory diseases or immunosuppressed patients (Moretti et al., 2018; Arrais et al., 2019).

The growth of biological species even after sanitation likely resulted from poor procedures. In addition, some factors may contribute to the persistence of these microorganisms, such as insufficient ventilation, resuspension of settled dust by sweeping the floor, water-damaged materials causing excess moisture, and the movement of people (Prussin and Marr, 2015). The prevention of airborne pathogens transmission is not simple process, it consists of a combination of measures, such as a control of airflow with the use of specially designed ventilation systems, added to use the practice of antiseptic techniques and wearing personalized protective equipment (Memarzadeh and Xu, 2012; Baseer et al., 2016).

Dehghan et al. (2018), when analyzing the concentration of bioaerosols before and after cleaning in operating rooms demonstrated the growth of bacteria above the recommended concentrations, and the growth of different species of fungi, even after sterilization and disinfection of the environment and HEPA filters. In this and others studies, the main fungal species found are *Aspergillus* sp. and *Penicillium* sp. (Perdelli et al., 2006; Dehghan et al., 2018; Zenaide-Neto and Nascimento, 2020). These filamentous fungi are associated with different negative health conditions, such as respiratory infections, skin lesions, allergies, among others (Egbuta et al., 2017). In addition, the toxigenic and cytotoxic potential of secondary metabolites produced by these and other fungi groups should be highlighted (Skóra et al., 2017).

The first hypothesis of the study is related to the fact that the conventional air conditioning system present in the health clinic favors the proliferation of bacteria and anemophilic fungi, in comparison with the air renewal system used in the judicial public sector. The second hypothesis of the study is that in both establishments, in some sectors, the presence of anemophilic fungi will occur above that permitted by technical norms.

In view of the growing concern with air quality in urban environments, indoor and outdoor, it is necessary to monitor air conditioning systems, both those that use the indoor air reuse system and those that use the system with air renovation. Thus, the objective of the study was to evaluate internal contamination in two different environments located in the city of João Pessoa, state of Paraíba, Brazil, which use different forms of air conditioning, analyzing the quantity and abundance of anemophiles fungal.

II. MATERIAL AND METHODS

Sampling sites

The study was carried out in two different types of location, a health clinic and a judicial public sector, located in the city of João Pessoa, state of Paraíba, Brazil. Both establishments are air-conditioned, in the clinic, a conventional system for reusing indoor air is used, and in the second establishment, the system used is for renovation with external air.

Collection and processing samples

The samples were collected inside of the establishments and data were provided for this study with permission and consent signed by their respective responsible.

The health clinic were divided into several environments, kitchen pantry, nursery, nursery for COVID-19, rest room, post-surgery room, operating room 01, operating room 03, operating room 04, medical Intensive Care Unit (ICU), neonatal ICU, and pharmacy ICU. The judicial public sector were divided in service location, cash machine, attendance boxes, nobreak room, and kitchen pantry.

Sampling was performed using the active method by air impaction. The equipment used in the sampling was a model of a 1-stage bioaerosol impactor, model CF-6 (Andersen type) that the human respiratory tract, more specifically the terminal bronchi (1.1 to 2.1 µm in diameter) characterized by sampling pump, flow rate: 28.3L / min, supply: 110V, dimensions 241 x 139 x 114mm and 3,880g in weight.

In operation, the impactor causes the flow to be collected through a surface filled with holes of predetermined diameter that prevents greater amounts of bacteria and fungi from 0.6 to 22 micrometers from reaching and contaminating the medium, affecting the flow speed of air and causing molecules to deviate. Thus, inert microorganisms collide with the culture medium of disposable Petri dishes that were fixed to the impaction system with culture medium ready for use (Fernandes 2014). The plates were identified according the location and the culture medium used was Agar Sabouraud Dextrose. The sampler was placed at a height of 1.5 meters and 70% alcohol was applied in the period between collections.

Microbiological analysis

The samples and microorganisms manipulation was carried out in the microbiology laboratory of the Department of Physiology and Pathology (DFP) located at the Health Sciences Center (CCS) of the Federal University of Paraíba.

The identification of the fungi used in the air samples was carried out by the slide microculture technique, which consisted of cultivation on microscopic slides in the humid chamber. For this, 0.5 cm² of Potato Dextrose Agar was transferred to the filtration center. With a flamed needle, each colony was picked by these fragments of the medium.

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A slide was added over the medium and incubated in a humid chamber, followed by the Petri dish lined with water-soaked paper. The incubation was performed in 3 to 5 days in an oven at 25 ° C. An answered microscopy of fruiting structures such as hyphae, conidia, and sporangiospores was performed with the aid of the addition of lactophenol blue dye (Carvalho 2018). For a macroscopic analysis of the colonies that isolate the primary isolation, characteristics such as color, texture, surface, and pigment dispersed in the culture medium were evaluated. To identify or fungus, a subculture was essential in Petri dishes containing Sabouraud Dextrose Agar, observed as formed reproductive structures. At the end of these analyzes, how dimensions were sterilized and discarded.

Data analysis/processing

With the quantitative and identification results, as microbial containers were verified according to the provisions of the norms 9 of 2003, RDC no. 15, of March 15, 2012, and DRC no. 222, of March 28, 2018, from ANVISA. Graph production were used using the GraphPad Prism 4.0.

III. RESULTS

Concentrations of airborne microorganisms

The **Table 1** demonstrated the values for the colony-forming unit (CFU/m^3) of fungal microorganisms. According to analyzes, nine of the eleven sectors in the health clinic were in poor condition regarding the total amount of fungal organisms. Analyzing the judicial public sector it was found that in two samples concentrations were recorded above the Resolution nº 9 of ANVISA (ANVISA, 2003).

Microorganisms groups

After counting of colony-forming units, the anemophilic fungal genera obtained from the samples were identified through microcultures for morphological analysis. In the **Figure 1** we can observed the genera found in the different sections of the health clinic, with a higher prevalence of detection of *Aspergillus* sp. and *Penicillium* sp., with the detection of *Aspergillus niger* (1%) in the kitchen pantry and in the operating room 01 (2%).

Figure 2 demonstrated the detection of fungal genera in the judicial public sector. *Aspergillus* sp. and *Penicillium* sp. were detected in all sectors, and as in the first location, *Aspergillus niger* (7%) was detected in the kitchen pantry. In this area it was possible to identify fungal genera different from the first, *Curvularia* sp. with 4% in the cash machine, and *Paecilomyces* with 9% in the attendance boxes.

Table.1: Concentration (Colony Forming Unit – CFU/m^3) of airborne fungi in different environments analyzed.

Designation	Colony number	CFU/m^3	I/E ratio	Class
Health clinic				
kitchen pantry	356	1,263	14.8	×
nursery	186	660	7.8	×
rest room	57	202	2.4	×
nursery for COVID-19	60	213	2.5	×
post-surgery room	42	149	1.8	×
operating room 01	59	209	2.5	×
operating room 03	45	160	1.9	×
operating room 04	52	184	2.2	×
neonatal ICU	64	227	2.7	×
medical ICU	26	92	1.1	↓
pharmacy ICU	6	21	0.3	↓
Judicial public sector				
service location	54	192	1.6	×
cash machine	72	255	2.1	×
attendance boxes	46	163	1.4	↓
nobreak room	41	145	1.2	↓
kitchen pantry	45	160	1.3	↓

Legend: × - samples that exceed the level of contamination established by ANVISA. ; ↓ - samples with contamination below the norm established by ANVISA.

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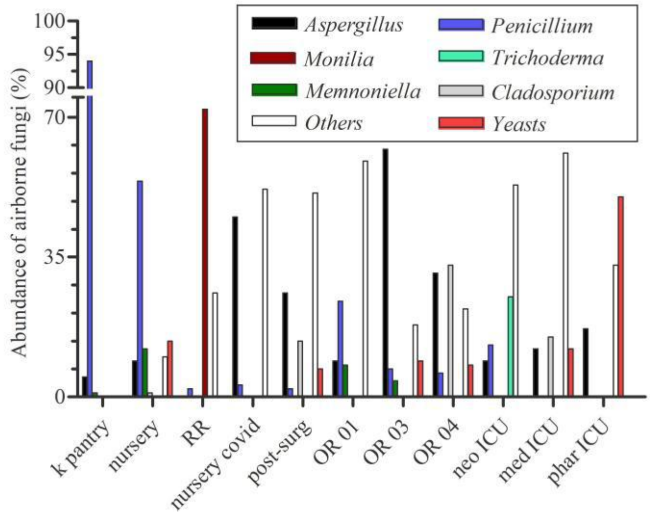

Fig. 1: Average percentage of fungal diversity in the interior rooms of a health clinic in Paraíba, Brazil.

Legend: RR – rest room; OR – operating room; neo ICU – neonatal ICU; med ICU – medical ICU; phar ICU – pharmacy UCI.

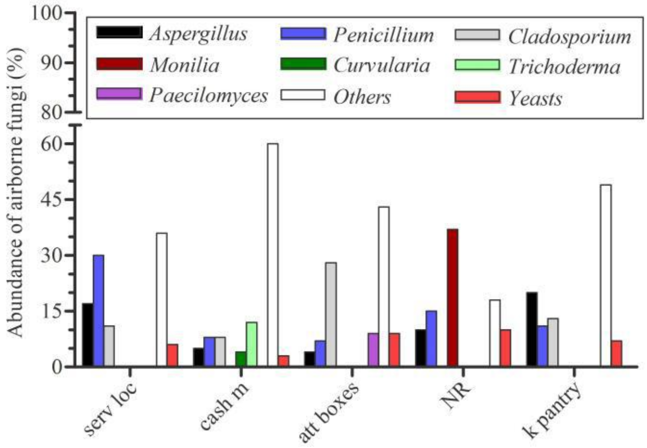

Fig. 2: Average percentage of fungal diversity in the interior rooms of a judicial public sector *in Paraíba, Brazil.*

Legend: serv loc - service location

;cash m – cash machine; att boxex - attendance boxes; NR – nobreak room.

IV. DISCUSSION

In this study, areas with different air conditioning system were evaluated. The clinic health use a conventional system, where the air already used is filtered and returned to the indoor environment. This is a less efficient air conditioning system than that using air renewal, with regard to minimization the amount of airborne pathogens. The clinic presented a greater contamination rate of airborne potential pathogenic and higher values of the *Penicillium* genus in comparison to the judicial public sector.

The quality of indoor air in a health environment is a critical factor for the health status of individuals, where an environment below the established quality standards can cause the aggravation of disorders and diseases already present in patients (Choi and Min, 2020). The highest fungal density was associated with the kitchen pantry (1,263 CFU/m^3) and the nursery (660 CFU/m^3), both with the majority detection of *Penicillium* sp. (94% and 54%, respectively), an organisms that can be an indicator of hospital indoor fungal levels (Araújo et al., 2008). In study of Zenaide-Nato and Nascimento (2020), *Penicillium* sp. was the genus detected in the highest percentage (40.72%) within a hospital located in the same city as the present study. *Penicillium* spp. is often found in indoor environments, and is present in high concentrations in homes with asthmatic children when compared to homes without an asthmatic child, being associated with the increase in exacerbated asthmatic conditions (Baxi et al., 2016).

Probably the higher rate of contamination of health clinic and the judicial public sector by fungal organisms may be related to failure in the cleaning of the physical space and to a greater circulation of people, where in most cases shoes are not sanitized after circulation outdoors. Fungi have excellent aerosol dispersion mechanisms in the atmosphere, however some measures such as cleaning the air conditioning system and water lines every two weeks can be effective in reducing the formation and dispersion of these bioaerosols (Oliveira et al., 2018).

The cooling system of the second location analyzed in this study, the judicial public sector, is made through the air renewal, where the external air is filtered before entering the internal environment. Some considerations must be made when using this system; first, it must be taken into account that the outside air directly influences the quality of the indoor air, since it has been demonstrated that the microbiota observed in indoor air were closely related with those in outdoor air (Prussin and Marr, 2015). Second, when there is a poor sanitization of the filter present in the air conditioning equipment, it is expected that in addition to microorganisms from outside, also microorganisms accumulated in the internal environment will contaminate the air (Fernstrom and Goldblatt, 2013).

Cash machine and service location were the sectors with the greatest abundance of fungal organisms, 255 CFU/m^3 and 192 CFU/m^3, respectively. This fact can probably be associated with a greater flow of people in these places, and consequently a greater abundance and microbial diversity. The species of anemophilous fungi found in the judicial public sector, can be potentially pathogenic and affect mainly those individuals who remain in this environment for a longer period of time, which is the case of employees. *Penicillium* sp., *Trichoderma* sp., *Cladosporium* sp., *Monilia* sp. and *Aspergillus* sp. were species were identified in higher percentages in certain sectors. The abundance of each of these genera varies practically according to the conditions of the environment, such as relative humidity and temperature (Segers et al., 2016). Continuous indoor exposure to fungal groups such as those mentioned above can become a risk factor for the development of asthma and increased asthma morbidity (Baxi et al., 2016).

The facts described demonstrated the main peculiarity of the clinic health environment in relation to the judicial public sector. The hospital is an environment of high selective microbial pressure, which favors the permanence of strains resistant to sanitizing products (Khan et al., 2018). In view of this aspect, preventive measures against the proliferation of microorganisms in these environments remains an effective air filtration system, with periodic filter changes, and restricted access for people in environments that deal with human health (Araújo et al., 2008). In addition to this, it is necessary to carry out qualified reports periodically about the sanitization of filters for air conditioning systems and the environment.

V. CONCLUSION

In this study, the anemophiles fungi levels were investigated in two different air-conditioned systems. Of the eleven sectors of the health clinic analyzed, nine had more contamination than the norm, including the operating rooms. In the judicial public sector, two sectors out of five exceeded the fungal contamination levels predicted by the norm.

The health clinic had a greater abundance of fungi than the judicial public sector, a fact probably related to the use of the conventional air conditioning system used in the first. The air conditioning system used in the second mentioned space tends to be more efficient than the conventional one, due to the renewal of the air that will be used indoor. The most common genera found in both environments were *Penicillium* sp. and *Aspergillus* sp.

The results presented in this study demonstrated the importance of regular and correct sanitization of filters and environment. Added to this, It is extremely important to periodically assess the quality of indoor air, in order to prevent the proliferation of potentially pathogenic organisms, both in hospitals or clinics and in environments with a greater daily flow of people.

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