

Air quality and microbiological control in a hospital in Paraíba, Brazil

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Abstract— An elevated quantity of pathogenic microorganisms can be an indicator of poor air quality, putting patient's health in hospitals at risk. The sanitation of the refrigeration systems must be carried out efficiently and with the right products, capable of maintaining reduced levels of hospital infection. In this work, the aim was to analyze the fungal density in the air of a private hospital, located in João Pessoa-PB, Brazil. The effectiveness of the disinfectant used to clean air conditioning systems in these environments was also verified. In an *in vitro* experiment with Thilex[®] disinfectant against common microorganisms in the hospital environment, the antimicrobial activity, concentration and time of action were evaluated. For the analysis of the air in refrigerated places in the hospital, a bio-aerosol impactor was used to quantify the pathogens. The antimicrobial test indicated that Thilex[®] was effective against *Klebsiella* sp., *Escherichia coli* and *Candida albicans*, while *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus* sp. and *Aspergillus* sp. presented resistance at the concentration of 2%, being controlled up to 20% of the product in distilled water and exposure time above 1 minute. The hospital's air samples indicated that 12 of the 23 rooms had a fungal density above the acceptable limit according to the current national regulatory standard, with a higher prevalence in obstetrics rooms. The most common genera were *Aspergillus* sp., *Penicillium* sp. and *Monilia* sp. These results indicate urgency in the development of more effective public policies in reducing the risk to patients exposed to low air quality in hospitals.

Keywords— Aeromicrobiology, Air conditioning, Anemophiles, Microbial control, *Penicillium* sp.

I. INTRODUCTION

Since the twentieth century, industries, homes, and hospitals have adopted the use of air conditioners to maintain indoor environments at a comfortable temperature (Afonso 2004). In urban centers, people spend about 90% of their time in environments acclimated by air cooling systems (Horve et al., 2019). It is expected that, with the increase in heatwaves around the world, the demand for the use of air conditioning systems in the long term may increase even more (Zuo et al. 2015).

These systems provide an ideal environment for the growth of potentially pathogenic microorganisms, such as fungi and bacteria, due to the high humidity rates and the accumulation of impurity in the devices (Hatayama et al. 2018). However, the diversity of bacteria and fungi residing in air conditioning filters and their possible health risks associated with hospitalized patients is not completely known (Acerbi et al. 2017).

The *split* system devices are the most used in healthcare centers in Brazil, due to their low cost,

durability, and easy maintenance (Marangoni et al. 2015). However, when the constant cleaning of the filters used in the equipment is hindering the growth of microbial bacteria. As these systems operate at high internal pressure, bacteria and fungi can be sprayed in the air and, therefore, they must consider the appropriate use of cleaning techniques and the replacement of these devices when necessary (Khare 2014).

Fungal infections occupy third place as the main cause of hospital infections and can have clinical manifestations that cause skin processes limited to generalized systemic infections (Blatzer 2017). Anemophilic fungi are microorganisms found dispersed in atmospheric air that can trigger allergic processes and cross-infections in immunocompromised patients, constituting the main contaminants in indoor air-conditioned environments (Calumby et al. 2019).

Immunocompromised patients belong to a group that may include people with hematological neoplasms, who have undergone transplants, with congenital immunodeficiencies and with the use of

immunosuppressive drug therapy (Martínez-Herrera et al., 2016).

It is a great interest in public health to minimize the risk of healthcare-associated infections (HAIs), especially in operating rooms. It is recommended that the microbial air reservoir be quantified through the analysis of samples, for which possible preventive measures, the quality of life of internalized patients is improved.

Anemophilic fungi and most pathogenic bioaerosols can disperse over great distances through the air and remain viable, increasing the possibility of infection even without contact with a source. Administrative Rules of the hospital protect vulnerable patients, maintaining strict airflow systems and isolating patients with infectious diseases transmitted by air in rooms that use HEPA filters in the piping. Despite these controls, the HAIs reaches an estimated 4.5 million deaths in Europe annually, leading to 37,000 deaths and adding 16 million additional days to hospitalized patients (Zingg et al. 2015). In Brazil, it is estimated that hospital infection rates reach 14% of hospitalized patients (ISC-UFBA, 2019).

The number of infections in surgical centers has increased in recent years, constituting a major health problem in Brazil, especially in Intensive Care Units (ICUs). These infections increase the length of hospital stay for patients and, consequently, increase the cost of hospital supplies, in addition to preventing bed rotation. Microbial resistance to antibiotics is one of the main causes of this and it can be intensified by poor air quality (Moura et al. 2018).

The control of internal microbial communities usually involves the removal of microbial biomass through physical and chemical agents. New infection prevention standards have been integrated into architectural designs and clinical procedures in healthcare facilities (Services 2004). Mechanical filtration, disinfection of cavities and walls of buildings, pressurization of rooms, and laminar flow have been implemented to control the spread of pathogens, but few studies confirm that they decrease HAIs rates (Tang 2009). This can be caused by inefficiency in control methods. Common surface cleaning techniques do not remove all microbial biomass from surfaces where, after just a few days, microbial communities are found even in cleanrooms or sterile reagents (Kwan et al. 2018). In addition, the use of cleaning materials contaminated by bacteria can be a source of contamination unable to remove them (Dharan et al. 1999). The use of powerful antimicrobial cleaning solutions can often contribute to a decline in adherence to hospital staff cleaning protocols

over time, leading to the persistence of microorganisms in public environments (Boyce et al. 2014).

Two hypotheses were used in this essay. The first is that the disinfectant used to disinfect air conditioners in the hospital environment is not efficient for controlling common microorganisms. The second, that the quantity of these pathogenic microorganisms may present a health risk for immunocompromised patients in hospital environments.

The work was divided into two stages: 1) Analysis of the *in vitro* antimicrobial test using the sanitizing product against the most common microorganisms detected in the hospital environment to verify the antimicrobial effect, and 2) Sampling and quantification of potentially pathogenic microorganisms present inside the hospital for comparison with current legislation.

The general objective was to analyze the risks of microbial contamination of the hospital environment, verifying the antimicrobial effect of the sanitizing product used in air conditioning systems against different airborne pathogens, and to evaluate the population and the diversity of fungi in the private hospital in the city of João Pessoa-PB.

II. MATERIAL AND METHODS

In vitro antimicrobial activity test

The manipulation of samples and microorganisms 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.

Seven microbial clinical isolates with pathogenic potential were used, some representative of the human microbiome and others present on surfaces, and normally transient in indoor air in hospital environments. The cultures were provided by the Lauro Wanderley University Hospital - HULW-UFPB and kept in the DFP/CCS laboratory. The species were identified as *Aspergillus* sp. and *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* sp., *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The growth and maintenance of the cultures were carried out in Agar Brain Heart Infusion (BHI) medium at 37 ° C for bacteria and Agar Sabouraud Dextrose at 25 ° C for fungi.

The Thilex® cleaning solution was aseptically diluted in sterile distilled water in the following concentrations: 2%, 20%, and 100% and the control with 0% (water only), making a final volume of 5mL in each

test tube. Then, a suspension of each previously cultured microorganism was standardized for turbidity based on the 0.5 McFarland scale tube and 1mL of this suspension was transferred to each tube containing the diluted solution. For each fungus, a suspension of around 10^8 CFU/mL was standardized.

After homogenization in a vortex mixer, 1mL aliquots were transferred in series. The tubes included as the product products were left to stand at different times (1, 10 and 20 minutes) at room temperature and after that, a swab was embedded in each tube containing the dilutions and seeded in Petri dishes using the culture medium suitable for growth, in triplicate (BHI culture medium for bacteria and Agar Sabouraud for fungi). After incubation at 36 ± 2 ° C for 48 hours (bacteria) and 25 ° C for 96 hours (fungi), the viability of microbial cells as specified by the growth responses on the plate.

Analysis of the microbiological quality of the hospital

This stage of the study was carried out in a private hospital, located in the city of João Pessoa, state of Paraíba, Brazil. The samples were collected inside and outside the hospital and data were provided for this study with permission and consent signed by the hospital's directory board.

The site area is characterized by a high concentration of people during its operation. As the hospital has a large area divided into several environments, the following areas were selected for sample collection: cafeteria, general ICUs, pediatric ICU, coronary ICU, obstetrics rooms, pre-delivery room, operating room, and center of materials for sterilization (CME). With the rooms contained in each area, there were 23 rooms in total. In addition to these internal environments, an area outside the hospital was also selected, as determined by the methodology described (Fernandes, 2014), established near the side entrance of the parking lot.

Sampling was performed using the active method of air impaction. In view of a large number of samples, sampling was carried out in two days, with daily sampling from an external location. The equipment used in the sampling was a model of a 1-stage bioaerosol impactor, model CF-6 (Andersen type) that simulates 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 a 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). As plates were identified with location and sample number 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.

The indoor air renewal process was monitored with an analysis of the concentration of CO² in parts per million (PPM) in active environments using a direct chemical reading device in each room. An analysis was carried out by a third-party company that provided the data. The parameter used was recommended by RE/ANVISA n° 9, with the maximum recommended value of carbon dioxide in the environment, being ≤ 1000 PPM, indicated for comfort and well-being.

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 a humid chamber. For this, 0.5 cm² of Potato Dextrose Agar was used. With a flamed needle, each colony was transferred to fragments of the medium. 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. At the microscope, it was possible to visualize fruiting structures such as hyphae, conidia, and sporangiospores with the aid of the addition of lactophenol blue dye (Carvalho 2018).

For a macroscopic analysis of the colonies, characteristics such as color, texture, surface, and pigment dispersed in the culture medium were evaluated. At the end of these analyzes, they were sterilized and discarded.

With the results of the identification and the quantitative tests, the 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.

Statistical analysis and graph production were used using the GraphPad Prism 8.0 and Microsoft Excel 2016 software.

III. RESULTS

In vitro tests

The microbial isolates originated from the University Hospital Lauro Wanderley - HULW-UFPB, in

the *in vitro* tests indicate that the microorganisms *Klebsiella* sp., *E. coli* and *C. albicans* were more susceptible to the disinfectant product, with no more growth in concentrations from 2 %, whereas *P. aeruginosa*, *S. aureus*, *Bacillus* sp. and *Aspergillus* sp. demonstrated susceptibility only from 20%.

Table.1: Growth of the pathogens in different concentrations and exposition time of the sanitizing product Thilex®

MICROORGANISM	PRODUCT CONCENTRATION AND EXPOSITION TIME					
	Control	2%	20% (1 min)	20% (10 min)	20% (20 min)	100%
BACTERIA						
<i>Escherichia coli</i>	+	-	-	-	-	-
<i>Staphylococcus aureus</i>	+	+	-	-	-	-
<i>Bacillus</i> sp.	+	+	-	-	-	-
<i>Klebsiella</i> sp.	+	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	+	+	-	-	-	-
FUNGI						
<i>Candida albicans</i>	+	-	-	-	-	-
<i>Aspergillus</i> sp.	+	+	-	-	-	-

Fungal quantification in the hospital

Analyzing the fungal community present in the hospital air in the 23 samples, it was found that in 12 samples concentrations were recorded above the ANVISA reference maximum limit, with the ratio of indoor air to outdoor air equal to or greater than 1.5. Fig. 1 indicates the indoor/outdoor ratio (or I/E ratio) between the sampling sites in an orderly manner. Environment classification parameter: internal sample / external sample: I/E ratio <1.5 = good; I/E ratio ≥ 1.5 = bad. The highest concentration of

fungi in the air (UFC / m³) was recorded (in descending order) in rooms 03, 05 and 01 of the obstetrics wards.

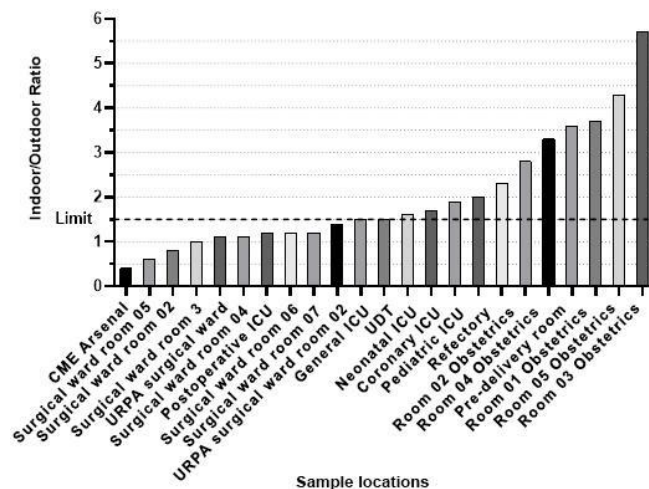


Fig. 1: Indoor/outdoor ratio values between sample locations at the private hospital in Paraíba, Brazil.

After incubation and counting of colony-forming units, the anemophilic fungal genera obtained from the samples were identified through microcultures for morphological analysis. Fig. 2 shows the genera most found in the hospital.

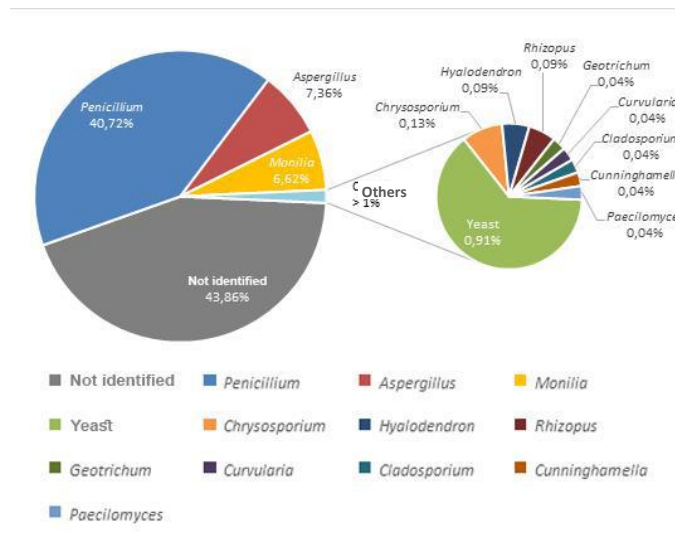


Fig. 2: Average percentage of fungal diversity in the interior rooms of a private hospital in Paraíba, Brazil.

The average percentage in all environments analyzed showed that among the identified genera, *Penicillium* with 40.72%, *Aspergillus* with 7.36%, *Monilia* with 6.62% and other less frequent genera predominated. However, most genera were not identified because they presented only the

formation of the mycelium, without reproductive structures.

There were two periods of collection to analyze the concentration of CO₂ in indoor environments. The concentration of CO₂ in indoor environments, according to the recommendations of RE/ANVISA n° 9, should not exceed the limit of 1000 PPM. Thus, when comparing the measured levels with the limits established by the legislation in force, as shown in the graph of figures 1, the operating room 05, Neonatal ICU and URPA reach values that exceed the maximum recommended value (MRV). The MRV is not exceeded in any of the other verified environments, providing a healthy environment for the occupants.

IV. DISCUSSION

A possible explanation for the resistance of pathogens to the disinfectant may be the presence of anionic surfactant in the composition of the product. Previous tests indicate that cationic surfactants are more effective in terms of antimicrobial effect, even when there is a synergistic resistance between bacteria (Patrone et al. 2010). As for the time of action, the product demonstrated effectiveness after 1 minute against all microorganisms tested.

Microorganisms such as *A. niger* and *P. aeruginosa* are studied as an application for bioremediation in sewage treatment plants because of their ability to not only resist but also degrade anionic surfactants. In one study, the presence of detergent caused a partial inhibitory effect on the growth of *A. niger* biomass (about 51.4%), however, the fungus demonstrated to degrade 30% of the anionic surfactant present in the medium after 16 days (Jakovljevic 2016). A *P. aeruginosa* strain showed degradation of about 96% of an anionic surfactant in the culture medium in 48 hours of incubation (Ambily 2012). This degradation capacity may have contributed to the findings of the current study, with *Aspergillus* sp. and *P. aeruginosa* possibly showing resistance to the anionic surfactant present in the product Thilex®.

That said, the sanitizing product used in air conditioning units may not have a direct influence on the variation in the internal fungal density of the hospital since its effectiveness has been within the expected standards.

To reduce bias that could interfere with the experiment, distilled and sterile water was used, which in fact does not reflect the composition of the water collected directly from the taps, as occurs in the cleaning process inside the hospital. Thus, future studies may use tap water,

considering the microbiota present, as well as correlate the effects on other pathogens not tested in this work, using different products and contact times. It is emphasized that the aseptic handling of microorganisms for the contact test reflected significantly on the repeatability and reproducibility of the assay and should be taken into consideration for future tests.

The highest fungal density was associated with the obstetrics and pre-delivery rooms, with *Penicillium* being the most commonly identified genus. This phenomenon can be attributed to the increase in dust, in favor of the deposit of fungal spores and probably due to an infrequent or insufficient cleaning of the environment. Surfaces that show greater contamination are the upper parts of furniture and the upper surfaces of large equipment (refrigerators, sterilization devices, heaters, etc.), where it is easier to detect or cause moisture damage (Brunetti et al. 2006).

It is necessary to observe the room that registered the lowest concentration of fungi during the study, which was the arsenal of the CMS. CMS is an acronym for Central of Materials and Sterilization or Center of Sterile Materials, and the objective is to be a sector dedicated to the cleaning, conditioning, sterilization and distribution of all medical articles in the hospital. ANVISA has established guidelines for the functioning of a CME, which must consist of: reception and cleaning room; preparation and sterilization room; chemical disinfection room (when applicable); area for monitoring the sterilization process; and a storage and distribution room for sterile materials. As support environments, provision should be made for: a dressing room with a bathroom for employees; cleaning material deposit; a pantry for sector employees; administrative room, and space for employees to rest during the night shift. A high inspection regarding the sterilization of the CMS room can serve as a model for indicating possible guidelines for use in rooms that have a higher fungus count, adapting to the prohibitions of each sector and each hospital (Tavares et al. 1979).

These results highlight the importance of environmental requirements and the need for cleaning procedures that can prevent fungal contamination in hospital departments. Immediate preventive action and specific training for the cleaning team, through education programs and application of infection control procedures, as well as corrective cleaning measures in contaminated rooms, will certainly have a directly detectable positive effect on the environment. The ideal time interval for cleaning air conditioners varies in different regions, but most protocols recommend sanitary maintenance of the unit every 7 to 15 days or at least twice a month (Aparecida 2011; Brenier-Pinchart 2009).

The installation of HEPA filters aims to eliminate biological contaminants from the air. According to a technical note from ANVISA, an inspection must be carried out periodically and the filter must be replaced when the differential pressure of the airflow or the passage reaches 45mmca or after 18 months of use, even if the differential pressure is less than 45mmca (Anvisa 2013).

Due to access limitations to the hospital, it was not possible to collect the samples before and after disinfecting and cleaning the devices. The study by Dehghani et al. (2018), carried out in a hospital unit in Iran, indicates that there can be a significant difference in the count of microorganisms before and after cleaning the rooms.

In that same study, 41% of the rooms had counts higher than the recommended values and the most prevalent microorganisms among the genera *Aspergillus* and *Penicillium*. Factors that influence the results, according to the researchers: low ventilation, a little wet variation of floors in the rooms, inadequate filtration of the air-cooling systems, high ventilation, and lack of ideal management of infectious patients after surgery. A recommendation for this case is to use HEPA filters, implementing more rigorous disinfection procedures, and improving or controlling temperature and humidity (Dehghani et al. 2018). Another study, carried out in a Portuguese hospital showed that, according to a fungal sampling in indoor air, the predominant genera were *Penicillium* spp. (41%) and *Aspergillus* spp. (24%) *Aspergillus* species were: *A. fumigatus*, *A. versicolor*, *A. glaucos* and *A. niger* (Cabo Verde et al. 2015). These results corroborate to those found in the present research, where there was a predominance of these two genders in all environments analyzed.

The results indicated that rooms with a high fungus count are worrying when in a scenario in which patients that suffer allergies are present. Sensitization to fungal allergens may be associated with allergic respiratory disease and atopic dermatitis. One study investigated a relationship between sensitization to different allergic genes, specific IgE sensitization rates for *Candida*, and was 81.2%, followed by *Aspergillus* in 69.2% and *Penicillium* in 63.2%, with lower values in patients with atopic dermatitis (Chang et al. 2010).

The fungal diversity found in the present study can be explained by the excellent mechanism of dispersion of fungi suspended in the atmosphere, which can be transported as bioaerosols over long distances with the movement of air. Certain fungi develop adaptations favorable to their survival while they are dispersed in the atmosphere, present in pollution, skin, tissues, and water droplets (Martínez-Herrera et al. 2016).

In general, physical-chemical conditions are not favorable to the growth of microorganisms and most can only remain viable until suspended for a short period of time. However, fungal conidia can propagate units with longer viability rates due to properties such as thick cell walls, which protect against desiccation and the pigment (melanin), that protects against ultraviolet radiation. Other important adaptations are thermotolerance and nutritional versatility, which allows the use of diverse sources of carbon and nitrogen, as in the case of *Aspergillus* conidia when they germinate (Abad et al. 2010).

Fungal genera can turn superficial infections into invasive microorganisms. Although fungi have several routes of entry into the host, the most common is the inhalation of propagules; therefore, maintaining good air quality is essential in critical areas of hospitals to reduce invasive fungal infections (Martínez-Herrera et al. 2016).

One of the most important diseases, in this case, is asthma, the most prevalent condition in the age between 0 and 18 years. In addition, pregnant women, ICU patients, and newborns under one year of age are more susceptible to these pathogens. The presence of fungi in the hospital can also be a risk factor for the health of workers and hospital employees (Abbasi et al. 2019). As seen in the present study, of the five ICU rooms, three of them were committed to levels above the acceptable level, that is, in bad conditions.

An external layer of spores (conidia) in fungi is rich in hydrophobin, which allows them to remain suspended without depositing, a cysteine present in the hydrophobin is highly active in the fungi surfactant. As the hydrophobins are organized in an amphipathic monolayer that reduces the surface tension of the medium or the substrate on which the fungus grows, it allows the interface to be below the water and avoid hydrosaturation to maintain gas permeability. The degree of hydrophobicity among fungi varies from a highly hydrophobic level, affecting the efficiency of the spore dispersal capacity (Bayry et al. 2012). A high density of sporulated fungi found in hospitalizations confirms a high dispersion capacity in the air and a high risk of contamination for patients.

The presence of *A. fumigatus* in similar studies was higher in hospital corridors. In the present study, a greater diversity of fungi was observed in the coronary ICU. The way this room was designed, containing a reception in the center and cabins with beds around it, can cause the traffic of people in a circular motion to be intensified and this can contribute to a greater spread of spores. Results presented in other studies may vary depending on the type of hospital, location, number of patients and visitors, climatic

conditions, geographic location, and laboratory conditions, such as incubation temperature and culture medium. As the hospital is located in the middle of the city and close to an urban road, the entry of more pollutants from vehicles may be common; Hospitals located in the metropolitan area, surrounded by vegetation and pastures have the lowest amount of pollutants (Abbasi et al. 2019). Although it has been argued that a source of fungal spores inside the building, including structures and construction conditions, fungal contamination can also be caused by colonies that grow on trees, plants, and shrubs and entrances inside through the door and window (Dannemiller et al. 2016).

Species of *Aspergillus* have pathogenic potential, with special attention to *A. fumigatus*, due to its conidia that are more hydrophobic than other species, in addition to giving more air suspension time, it has the ability to hide the cell wall from inducing a response immune to the host. The hydrophobin RodA protein presents as a virulence factor that prevents the immune system recognition and the recruitment of neutrophils and the production of cytokines. Disinfection using products composed of hydrofluoric acid removes the RodA hydrophobin from the micro-organism and can decrease its pathogenic action. However, pathogenicity can be intensified in immunocompromised patients, making it impossible to generate an immune response and the risk of infection (Carrion et al. 2013).

The third most common fungal genus in this study was *Monilia*, with a higher prevalence in the postoperative ICU. These fungi are anamorphic, being a reproductive phase of *Monilinia* (Andrade 2016). They are not known as human pathogens, being more associated with diseases in angiosperm plants such as *Rosacea* and *Ericaceae*, in addition to causing fruit rot (Hu et al. 2011). Some species of the genus *Monilia* are identified as residents of the human intestinal microbiota and their clinical relevance is more useful for biological indicators of diseases, being observed in small numbers in the intestines of patients with chronic inflammatory diseases, such as Crohn's disease (El Mouzan et al. 2017).

Some yeast-like genera were found in the hospital's rooms. As stated earlier, *Candida albicans* is a predominant cause of invasive yeast infections; however, epidemiology of yeast infections is gradually evolving and other rare yeasts have emerged as life-threatening opportunistic pathogens. *Trichosporon* spp., the second or third most common cause of yeast infection, demonstrated less susceptibility to amphotericin B and 5-flucytosine and resistance to echinocandins (Guo et al. 2017). These findings reinforce the need to monitor the presence of invasive yeasts and antifungal resistance among the variations of regular yeasts today and in the future.

Regarding the concentration of CO₂ in the indoor environments analyzed in this study, the rooms with lower levels of air recovery (higher concentration of CO₂) do not correspond to a high concentration of fungi found. Like the neonatal ICU rooms, the operating room 5 and URPA showed values above the ANVISA VMR. Of these rooms, only the neonatal ICU has shown a high fungal count (I/E ratio > 1.5), and the room that shows the highest concentration of fungi, obstetrics room 3, has one of the lowest values of CO₂ concentration is compared to the other rooms.

There are studies in the literature that correlate the concentration of CO₂ with the increase of fungal concentration in indoor environments. This relationship may be more indirect, since it may indicate a high standard of human activity in the analyzed internal environment. As the rates of generation of CO₂ and bio-effluents depend on human activity, the concentration of CO₂, and the intensity of human bio-effluents in space exhibit a relationship related to the number of occupants. Therefore, the use of CO₂ concentration to monitor indoor air quality may be more appropriate to demonstrate the human population density of an indoor or outdoor environment (Chaivisit et al. 2018).

In short, fungal contamination is directly related to air quality, which is a major contributor to the transmission and diffusion of fungal spores. To reduce contamination, special equipment should be used for patients at risk, excessive human movement should be avoided in hospital corridors and windows should be closed. In addition, performing systematic disinfection of air conditioning systems, as well as periodic monitoring of the fungus population, which are indicators of environmental quality. One of the measures may be the use of HEPA filter in cooling systems, which easily and efficiently capture fungal spores and help to isolate environments used as operating rooms.

The results show that the environmental monitoring of biological indicators is an important tool and should be adopted by hospital infection control committees to investigate, control and reduce the occurrence of infections, contributing to reducing the economic impact on hospital admissions.

V. CONCLUSION

A high amount of pathogenic fungi is present at-risk sites in the private hospital located in João Pessoa-PB, Brazil. Of the 23 rooms analyzed, 12 had more fungi density than permitted by the norm, including obstetrics, pre-delivery, cafeteria and pediatric, coronary, and

neonatal ICUs. The most common genera found were *Penicillium* (40.72%), *Aspergillus* (7.36%), and *Monilia* (6.62%), however, most of the examples were not identified. The *in vitro* antimicrobial tests of the sanitizing product used in cleaning air conditioning devices (Thilex®) showed that *Klebsiella* sp., *E. coli* and *C. albicans* are susceptible and *P. aeruginosa*, *S. aureus*, *Bacillus* sp. and *Aspergillus* sp. are resistant to less than 2% of the product in distilled water, with the most suitable usage instructions being 20% of the product concentration and exposure time above 1 minute.

Adequate management of patient admission and visit time can be effective in contaminating indoor environments in hospitals, such as the use of protective filters for rooms with immunocompromised patients. In addition, the control of these parameters can prevent health and psychometric problems for health professionals in the long term.

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