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FUNGAL BIODIVERSITY OF AIR IN HOSPITALS IN THE CITY OF FORTALEZA, CEARÁ, BRAZIL

Biodiversidade fúngica do ar de hospitais da cidade de Fortaleza, Ceará, Brasil

Artigo Original

ABSTRACT

Objectives: *To monitor the environment in specific areas of three tertiary hospitals in Fortaleza - CE, seeking to report the presence of potentially pathogenic fungi for patients and staff, contributing to a better risk assessment in these hospitals.* **Methods:** *In the period from December, 2005 to November, 2006, air samples from three public hospitals were collected monthly, which resulted in 180 air samples originated in 15 hospitals. The biological specimens were collected using the passive method of sedimentation, with exposure of Petri dishes containing Sabouraud agar supplemented with antibiotic. The dishes were incubated for 10 days (28°C) and all fungal colonies developed were subsequently identified.* **Results***: 10,608 colonies were isolated, belonging to 16 genera, the most common being Aspergillus, Penicillium, Candida, Curvularia and Trichoderma. There were no statistically significant relationships between the total number of colonies and the characteristics of each environment studied, except for three of those.* **Conclusion:** *The difference in fungal concentrations in the air of these hospitals is possibly more related to instability of human activities, such as overpopulated settings and construction works, than to climatic variations observed in the period.*

Descriptors: *Environmental Monitoring; Fungi; Hospitals.*

RESUMO

Objetivos: Monitorar o ambiente em áreas específicas de três hospitais terciários da cidade de Fortaleza – CE, buscando relatar a presença de fungos potencialmente patogênicos para pacientes e funcionários, contribuindo para uma melhor avaliação de riscos nesses hospitais. **Métodos:** No período de dezembro/2005 a novembro/2006, foram realizadas coletas mensais de amostras do ar de três hospitais públicos, que resultaram em 180 amostras de ar oriundas de 15 ambientes hospitalares. Os espécimes biológicos foram coletados através do método da sedimentação passiva, com exposições de placas de Petri, contendo ágar Sabouraud, suplementado com antibiótico. As placas foram incubadas por 10 dias (28°C), com a posterior identificação de todas as colônias fúngicas desenvolvidas. **Resultados:** Isolaram-se 10.608 colônias, pertencentes a 16 gêneros, sendo os mais frequentes *Aspergillus, Penicillium, Candida*, *Curvularia* e *Trichoderma.* Não se observaram relações estatisticamente significativas entre o número total de colônias e as características de cada ambiente analisado, com exceção de três ambientes. **Conclusão:** A diferença na concentração fúngica do ar desses hospitais possivelmente está mais relacionada com desequilíbrios das atividades humanas, tais como ambientes superpovoados e obras de construção, do que com as variações climáticas observadas no período em análise.

Descritores: Monitoramento Ambiental; Fungos; Hospitais.

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INTRODUCTION

The importance of bioaerosols has been emphasized in recent decades due to their possible effect on human health, leading to conditions ranging from allergies to disseminated infections in susceptible patients[1-3]. Different authors have reported the importance of these particles as causative agents of nosocomial infections[4,5], especially fungal isolates, since they act as epidemiologic markers of microbial contamination[6]. Fungal infections of hospital origin have been gaining importance in recent years due to their progressive increase and the high rates of morbidity and mortality with which they are associated[7-9].

When fungal proliferation occurs, aerospores are abundantly distributed on surfaces and in the air, so indoor environments become a source of exposure to occupants. Knowledge of indoor environmental mycoflora is especially important from an allergologic standpoint because in many cases it differs from that observed in outdoor environments[5].

The filamentous fungi are frequently found in air and have an important adaptation of their physiology to the environment, especially the ones belonging to *Aspergillus, Cladosporium, Paecilomyces, Penicillium* and *Scopulariopsi* genera[10,11]. Yeasts of *Candida*[7], *Rhodotorula*[5] and *Trichosporon*[12] genera also present this characteristic. All the mentioned genera are described as potential human pathogens[10].

Bioaerosol monitoring in hospitals can provide information for epidemiological investigation of nosocomial infectious diseases, research into the spread and control of airborne microorganisms and as a quality control measure[13]. Therefore, the purpose of this study was to monitor the fungi in specific areas of three local tertiary hospitals.

METHODS

The present study was conducted in three tertiary hospitals in the city of Fortaleza, Ceará, Northeast Brazil (Hospital A, Hospital B and Hospital C).

The hospitals chosen are reference institutions for the treatment of patients with immunosuppression of any nature, such as patients suffering from cancer and infectious contagious diseases and transplant patients. Some particular characteristics of each one are described below:

Hospital A. Tertiary care in the area of pediatrics including cancer, with humanized care for the child and parents.

Hospital B. Tertiary care, serving the city and outlying areas, specialized in high-complexity services.

Hospital C. Philanthropic institution noted for excellence in the prevention, diagnosis and treatment of cancer as well as cancer research and teaching.

The air sampling occurred during 12 months (December 2005 to November 2006). All told, 180 Petri dishes were analyzed from five environments from Hospital A, Hospital B and Hospital C, considering two types of air microbiota: open environment (patio) and closed environment (reception area, consulting room, ambulatory ward and intensive care unit), along with the peculiar characteristics of each environment.

Sample collection was performed using the passive sedimentation method in Petri dishes (150 mm in diameter), containing Sabouraud dextrose agar medium (Sanofi®, France) supplemented with chloramphenicol and then incubated at 28°C for ten days. The dishes were exposed into each environment for 12 hours (from 08:00 a.m. to 08:00 p.m.) and positioned two meters high – roughly human respiration height[14]. The placement of the dishes into each environment depended on the characteristics of each one, but most were placed on the top of a cabinet as centrally located as possible. The Petri dishes were then sealed and sent to the State University of Ceará Microbiology Laboratory.

During the exposure period, the environments (with either natural or artificial air conditioning) were monitored with a calibrated thermo-hygrometer to read the highest and lowest temperatures. Rainfall data was provided by Ceará Foundation for Meteorology and Water Resources – FUNCEME.

After colony counting, a triage based on macroscopic characteristics was performed, to isolate all possible genera in each Petri dish. The chosen colonies were subcultured in tubes with potato dextrose agar (Himedia®, India), to purify the colonies and enhance the identification.

Filamentous fungi were identified by macroscopic and microscopic examination of the fungal colonies' characteristics with the aid of identification keys[10] and yeasts were identified according to their morphological characteristics, biochemical profile and growth in differential culture media[15].

The data was analyzed by descriptive statistics, analysis of variance and Tukey test to compare mean values.

This study was submitted to analysis by research ethics committees of the three institutions and obtained consent in September 2005, protocol number 05202041-0.

RESULTS

Overall, there were 10,608 fungal colonies counted from all the environments, with 4,447 colonies isolated from Hospital C, followed by 3,644 and 2,517 from Hospitals B and A, respectively.

Comparison of the colonies counted from different hospital environments showed a significant statistical relation in one environment from each hospital: the patio of Hospital C, reception area of Hospital B, and patio of Hospital A (Table I).

Table I - Difference between colonies counting mean values, in different environments from Hospitals A, B e C, in the period of December/2005 to November/2006.

Environments	Hospitals		
	A	B	C
Patio	2.84 a	49.0 b	172.5 a
Intensive Care Unit	43.75 b	73.67 b	50.17 b
Ambulatory Ward	54.34 b	45.84 b	63.33 b
Consulting Room	58.17 b	36.08 b	34.41 b
Reception Area	50.67 b	99.08 a	50.16 b

a- Mean values followed by the same lower case letter within a column are not significantly different ($p<0.05$).

Table II. Absolute frequency distribution of the fungal genera identified for each hospital and environment analyzed, from December/2005 to November/2006.

Hospital	Environments	Fungal General															
		Acremonium spp	Alternaria spp.	Aspergillus spp.	Candida spp.	Chrysosporium spp.	Cladosporium spp.	Curvularia spp.	Fusarium spp.	Mucor spp.	Penicillium spp.	Rhizopus spp.	Rhodotorula spp.	Scopulariopsis spp.	Trichosporon spp.	Trichoderma spp.	Mycelia sterilia
A	PAT	1	-	-	-	-	-	-	-	1	1	-	-	1	-	-	-
	ICU	1	2	7	9	-	1	4	-	1	12	2	1	2	-	8	1
	REC	1	-	12	7	-	2	5	-	2	10	2	1	3	-	5	1
	AMB	1	-	10	9	-	2	3	1	-	9	-	2	1	-	8	-
	CON	1	1	9	9	-	-	10	-	1	12	-	4	-	-	8	1
B	PAT	2	1	6	2	-	-	1	-	-	5	-	1	1	-	-	1
	ICU	3	-	12	6	-	1	5	3	2	10	1	1	1	-	3	-
	REC	6	-	10	5	-	2	2	1	1	9	-	1	4	-	5	-
	AMB	5	1	11	3	-	1	3	1	-	8	1	2	4	-	10	1
	CON	2	2	10	5	-	3	3	1	-	8	-	2	3	-	6	-
C	PAT	2	-	10	-	-	1	3	4	1	10	2	-	-	-	6	-
	ICU	3	1	9	8	1	-	4	-	-	8	-	2	3	-	3	-
	REC	3	1	7	9	-	2	3	1	-	6	-	1	1	-	-	1
	AMB	4	-	9	9	-	1	2	-	-	11	-	2	1	-	3	-
	CON	1	-	5	6	1	3	4	-	-	11	-	3	1	1	2	-
	TOTAL	36	9	127	87	2	19	52	12	8	130	8	23	26	1	67	6

Legend: PAT: Patio; ICU: Intensive Care Unit; REC: Reception; AMB: Ambulatory Ward; CON: Consulting Room. a- Order Agonomycetales.

Concerning qualitative analysis, 16 fungal genera were isolated, with predominance of *Penicillium*, *Aspergillus*, *Candida*, *Trichoderma* and *Curvularia*. Hyphomycetes that could not be identified to the genus level were included in the order *Agonomycetales* (*Mycelia sterilia*) (Table II).

Rainfall during the study period fluctuated between a maximum of 381.5 mm (rainy season) and a minimum of 110.3 mm (dry season). Despite the big difference between maximum and minimum precipitation and the different data obtained by the thermo-hygrometer in each hospital environment, there were no significant statistical relations ($p \geq 0.05$) regarding the number of colonies.

DISCUSSION

Periodic environmental monitoring in different hospital areas is important, since bioaerosols can be rapidly transmitted by air, acting as a source of infectious agents[16].

Air particles can have many origins[17]. For example, in environments with artificial ventilation, the air-conditioning system, due to condensation trays, has been considered an important source of microorganisms[18]. However, in environments with natural ventilation, the main origins of air particles are the fans, nebulizers, air humidifiers, plant vases, some foods and people themselves[4].

The high number of colonies (n = 2,070) observed in the patio of Hospital C was probably due to the construction work it was undergoing to expand one of its wards. It is known that the existence of an external source of bioaerosols, such as humid construction material, provides ideal conditions for microorganism proliferation[19].

Unlike Hospital C, the patio of Hospital A was the environment with the lowest number of colonies (n = 34). This environment is surrounded by wards and is used as a recreation area, with high incidence of sun light and higher temperature. This possibly affected the culture media quality during the exposure period of the Petri dishes, causing some dishes to be free of contamination.

At Hospital B, the reception area had the highest number of colonies (n = 1,189). This environment is an access to emergency ward for patients assisted under the National Health System, characterized by a great number of transients, with low ventilation and high temperatures.

Concerning the composition of airborne fungi from the three analyzed hospitals, it consisted predominantly of hyaline filamentous deuteromycetes. According to various authors, the *Aspergillus* genus is one of the main components of fungal air microbiota[10,20]. This is cause for concern and increases the need of periodic monitoring of hospital air, since some members of this genus, particularly the species *A. fumigatus,* are known as agents of opportunistic infections.

The only representatives of the yeast group were the genera *Candida* and *Rhodotorula,* found at all three hospitals, and the genus *Trichosporon,* present only at Hospital C. Aerobiological studies performed in temperate countries have indicated dematious fungi, especially the genus *Cladosporium*, as the preponderant members in air and dust[21]. In the present study, the diversity of the dematious fungi was low, represented only by the *Alternaria*, *Curvularia* and *Cladosporium* genera.

The results of this study on the relationship between the presence of fungi and climatic factors differ from those found by some other authors, who often report a direct relationship between the number of fungal colonies and the season[21,22]. Differences in fungal distribution and quantification related to seasonality were not possible to establish. This can probably be explained by the absence of well defined seasons in the state of Ceará, which is near the Equator: the air temperature averages around 25 and 35ºC throughout the year. Although there is a dry season (June to November) and a rainy season (December to May), the difference in average monthly rainfall levels is not great (average monthly rainfall of 381.5 mm in the wet season versus 110.3 mm in the dry season).

In recent years, opportunistic fungal diseases have been increasing, along with the diversity of the isolated fungi and severity of the infections caused. The hospital environments analyzed presented similar contamination levels, especially with respect to the quantitative analysis of the *Aspergillus* genus. Therefore, considering the presence of these microorganisms with high pathogenic potential and the compromised immunity of many of the patients, air monitoring is essential to help prevent hospital infections.

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