Monitoring of airborne fungus and yeast species in a hospital unit

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Fungi. Yeasts. Air conditioning. Cross infection, prevention and control. Aerosols. Infection control.

Abstract

Objective
To monitor and characterize airborne filamentous fungi and yeasts from abiotic and biotic sources within a hospital unit.
Methods
Collections were carried out on a monthly basis, at two different time periods, from the adult and pediatric intensive care units and surgical center of a hospital in Araraquara, Southeastern Brazil. Collection of airborne fungi was carried out using a simple-stage Andersen sample. The presence of yeasts was investigated in samples taken from the hands and oropharynx of staff members as well as from the surface of beds and doorknobs inside the critical areas.
Results
Thirty-two genera of airborne fungi and were recovered from the surgical center and 31 from the intensive care units. Genera most frequently isolated were *Cladophialophora* spp., *Fusarium* spp., *Penicillium* spp., *Chrysosporium* spp. and *Aspergillus* spp. During the study period, a new unit was built in the hospital, which coincided with an increase in *Cladophialophora* spp., *Aspergillus* spp., and *Fusarium* spp. colony counts. Yeasts were found in 39.4% of samples obtained from healthcare staff (16.7% from interdigital spaces, 12.1% from nailbeds, and 10.6% from oropharynx) and in 44% of furniture samples, with a predominance of the *Candida* genus (*C. albicans, C. guilliermondii, C. parapsilosis* e *C. lusitaniae*), followed by *Trichosporon* spp.
Conclusions
We found a relatively high number of airborne fungi (potentially pathological) in special areas and expressive levels of yeasts in both biotic and abiotic samples. Microbiological and environmental monitoring should be conducted, especially in special areas which include immunocompromised patients, who are more susceptible to the exposure to environmental and staff-derived pathogens.

INTRODUCTION

The importance of bioaerosols has been emphasized in the last decades due to their relationship to human health, leading to the appearance of diseases that range from allergies to disseminated infections in susceptible patients.[5] Determining the composition and concentration of airborne microorganisms in internal and/or external areas of critical hospital wards is considered as an extremely necessary measure.[1] Hospitals are environments that require great care in terms of the environmental monitoring of critical areas. The aim of such a measure is to identify possible sources of contamination and dissemination, as well as the etiological agents potentially involved. On the other hand, in climate-controlled environments, the accumulation of humidity and organic matter inside air-conditioning trays may

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render them powerful sources of dispersion of bioaerosols.

Fungal infections of hospital origin are gaining in importance in recent years due to their progressive increase and to the high rates of morbidity and mortality with which they are associated.[4,19] Many of these infections are endogenous in nature, but others can be acquired by exogenous routes, through the hands of healthcare workers, contaminated infusion products and biomaterials, and abiotic environmental sources.[19,25] Monitoring bioaerosols and the microbiota of adjacent areas and hands of healthcare professionals may provide epidemiological information regarding the microorganisms involved in nosocomial infections.[8,25]

Thus, in the present study, we carried out the environmental monitoring of hospital areas, isolating and quantifying both fungal bioaerosols and yeasts from abiotic sources and healthcare workers.

METHODS

The present study was conducted in critical areas of a hospital in the city of Araraquara, Southeastern Brazil, with monthly collections between October 2001 and August 2002. Critical areas included adult and pediatric intensive care units (ICUs) and surgical center, all of which are climate-controlled by air-conditioning and/or ventilation, but which lack HEPA (high-efficiency particulate air) filters. Collection of air samples from the critical areas was carried out at two different times, one during the morning, immediately following cleaning, and another during late afternoon, at the end of the regular shift.

Sampling totaled 196 air samples from critical areas of the hospital (surgical center and adult and neonatal ICUs) collected using appropriate equipment, following the recommendations of Andersen.[16] Samples from the access routes to the critical areas and from a spot outside the building were collected on the same days as the sampling of internal areas.

Following the results of a pilot-test, airborne fungi were isolated by seeding the contents of 80 liters of air on Sabouraud agar + chloramphenicol culture medium. The identification of airborne fungi was based on the association of macroscopic aspects and microscopic characteristics of primary cultures. Identifications were later confirmed by sporulation characteristics following microculture on Lactrimel medium.[10,11]

Simultaneously, the presence of yeasts was investigated on the hands, nails (interdigital spaces and nailbeds), and oral mucosa of 66 professionals directly in contact with ICU and surgical-center patients. Ninety-one swab samples were collected from doorknobs, beds, and telephones located inside the ICUs and surgical center.

Yeasts from biotic and abiotic sources were isolated on Sabouraud agar + chloramphenicol culture medium. Yeasts were identified previously on CHROMagar Candida medium. These results were associated to filamentation, germ-tube formation after two hours, assimilation and fermentation of carbon and nitrogen sources for confirmation. Identification was based on Kurtzman & Fell[9] and Mendes-Giannini & Melhem.[12]

Kruskal-Wallis, Mann-Whitney, Anova-Manova, and *t*-tests were performed in order to analyze variables associated to collection time, location, and month of isolation, as well as the prevalence among the isolated genera. Statistical analyses were performed using Biostat 3.0 software.

RESULTS

Potentially pathogenic and toxigenic fungi were isolated from both environment and staff (Table). Thirty-one and 32 genera were isolated from surgical center and ICUs, respectively. Quantitative analysis of colony counts at the two collection times was relatively similar in the ICUs, but not in the surgical center ($p<0.05$). *Cladophialophora* was the predominant genus during morning (59.2%) and afternoon (74.4%), with significant differences ($p<0.05$) between collection times and during certain months.

We obtained 33,317 CFU/m^3 in the ICUs, with a mean 317.1 CFU/m^3 for internal environments and 454.6 CFU/m^3 for environments outside special areas but inside the building. From the surgical center, we isolated 34,301 CFU/m^3, with a mean 332.2 CFU/m^3 from internal environments and 482.8 CFU/m^3 from external ones. Forty-six samples were collected from locations outside the adult and pediatric ICUs and the surgical center, totaling 10,487 CFU/m^3 and 4,401 CFU/m^3, respectively. From the environment outside the building, we isolated 5,388 CFU/m^3 from eight samples, with a mean 673.5 UFC/m^3.

In the surgical center, *Cladophialophora* spp., *Fusarium* spp., *Penicillium* spp., and *Aureobasidium* spp. were present in 10 of the internal points evaluated. *Fusarium* spp. were isolated from all rooms during the morning period and from nine of 10 points in the afternoon collection. *Aspergillus* spp. were found in four of 10 points during the morning period.

Table - Airborne fungi isolated from surgical center and intensive care units during different time periods.

	Ocurrence of fungi		Total colonies in the environment (UFC/m³)			
Genus	SC	ICU	SC - M	SC - A	ICU- M	ICU- A
Cladosporium spp.	+	+	6,338	16,587	11,587	11,192
Fusarium spp.	+	+	2,350	900	514	612
Penicillium spp.	+	+	912	813	1,425	950
Chrysosporium spp	+	+	401	562	637	950
Aspergillus spp.	+	+	362	289	775	413
Aureobasidium spp.	+	+	562	200	238	476
Mycelia sterilia	+	+	350	300	64	237
Trichoderma spp.	+	+	325	100	62	250
Monilia spp.	+	+	89	275	162	175
Paecilomyces spp.	+	+	262	200	26	75
Curvularia spp.	+	+	162	13	75	212
Rhodotorula spp.	+	+	200	138	38	63
Chaetomium spp.	+	+	275	12	137	–
Stemphylium spp.	+	+	162	100	87	38
Rhinocladiella spp.	+	+	75	38	88	150
Exserohilum spp.	+	+	25	–	201	125
Epicoccum spp.	+	+	–	75	137	88
Phoma spp.	+	+	100	25	13	100
Alternaria spp.	+	+	26	26	137	25
Nigrospora spp.	+		162	51	–	–
Syncephalastrum spp.	+	+	51	87	37	37
Bipolaris spp.	+	+	25	25	87	26
Dactylaria spp.	+	+	12	–	12	112
Acremonium spp.	+	+	25	12	87	–
Conidiobulus spp.		+	–	–	87	37
Verticillium spp.	+	+	12	–	100	–
Gliocladium spp.	+		62	37	–	–
Pithomyces spp.	+	+	–	25	25	12
Sepedonium spp.		+	–	—-	50	–
Scopulariopsis spp.	+	+	–	25	12	–
Sporotrichum spp.	+	+	–	12	25	–
Ulocladium spp.	+	+	–	12	–	25
Scedosporium spp.	+		25	–	–	–
Emonsia spp.		+	–	–	–	12
Geotrichum spp.	+		12	–	–	–
Total	32	31	13,362	20,939	16,925	16,392

SC: Surgical center; ICU: Intensive care units; M: Morning; A: Afternoon

Fusarium spp. were prevalent ($p<0.05$) in the month of March during the morning; in May, *Aspergillus* spp. were also isolated at similar levels to the dematious fungus, whereas, in the remaining months, there was a prevalence of *Cladophialophora* spp. This genus increased significantly ($p<0.05$) in the month of March during the afternoon, falling gradually in subsequent months (Figure 1).

In the ICUs, over 60% of isolates from all rooms were of the *Cladophialophora* genus. There was a correlation between the number of colonies and the number of occupied hospital beds in both collection periods. *Fusarium* spp. were found in the five rooms, whereas *Aspergillus* spp. were found in four. In the adult ICU, during the morning period, *Cladophialophora* spp. remained stable during the months of October, December, and February, began to increase in April, and peaked in July ($p<0.05$). The same occurred in the afternoon period, but counts were proportionally lower, and differences between the two periods were significant ($p<0.05$) in October, February, and July (Figure 2).

In the Neonatal ICU (Figura 3), *Cladophialophora* spp. prevailed in July ($p<0.05$) and April ($p<0.05$), in the morning and afternoon periods, respectively, with a subsequent reduction in counts.

During renovation, there was a significant difference ($p<0.05$) in airborne fungus counts in relation to the sampled months. Higher rates of the predominant genus were found in external environments when compared to internal ones, especially during the afternoon (data not shown).

Another aspect of the survey focused on the healthcare staff of the adult and neonatal ICUs and of the surgical center. Yeasts were isolated from 39.4% of staff members. From nailbeds 12.1% of samples were isolated, 16.7% from interdigital spaces, and 10.6% from the oropharynx.

Of the positive samples obtained from staff members, *Candida albicans* accounted for 23%, *C. parapsilosis* for 19%, *Candida* spp. for 19%, *C. guilliermondii* for 8%, and *Trichosporon* spp. for 31%. *C. albicans* was isolated mainly from the oropharynx and *C. parapsilosis* and *Trichosporon* spp, from interdigital spaces and nailbeds.

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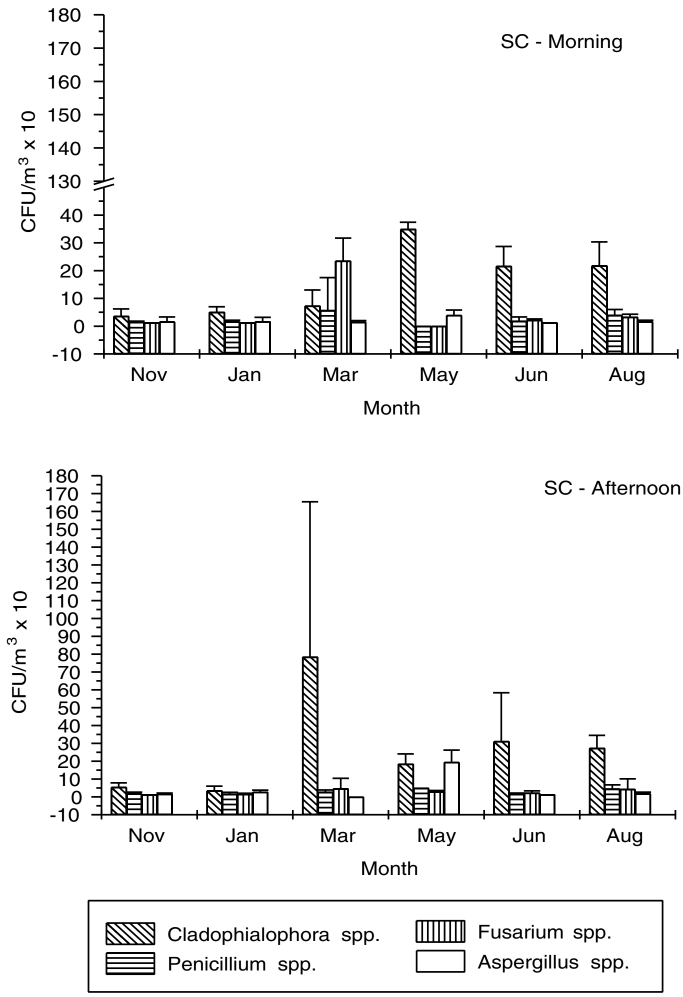

Figure 1 - Major genera isolated from the surgical center, in CFU/m^3 (geometric mean ± standard deviation), during morning and afternoon collection periods in the months of November, January, March, May, June and August.

A total 91 samples was obtained from furniture samples, of which 44% were positive for the presence of yeasts. *Candida* was the predominant genus (70%), followed by *Trichosporon* spp. *Candida guilliermondii* prevailed in the environment, especially on doorknobs (52%), where *C. lusitaniae* (5,0%), *C. parapsilosis* (3,0%), *Candida* spp. (10%), and *Trichosporon* spp. (30%) were also isolated. *Trichosporon* spp. were isolated from all the environments investigates, prevailing on doorknobs and in the air inside the ICUs.

DISCUSSION

The incidence of fungi is widely variable, depending on season, temperature, relative humidity, time of the day, speed and direction of winds, presence of human activity, and artificial climate control.[6,11,13]

The composition and concentration of airborne organisms in external and internal environments of critical hospital areas have been little investigated, but there are studies that emphasize their importance due to the appearance of these agents in nosocomial infections.[1]

In the present study, about 30 different genera were isolated from a hospital environment, with a predominance of *Cladophialophora* spp., *Fusarium* spp., *Penicillium* spp., *Chrysosporium* spp., and *Aspergillus* spp. The number of studies addressing this subject in Brazil is limited, and data on the airborne microbiota in hospitals are sparse. Silva et al[22] investigated the fungal microbiota of the air and floor of a hospital, and found mainly *Cladosporium* spp. (65.0%), *Aspergillus* spp. (37.1%), *Mycelia sterilia* (26.9%), *Fusarium* spp. (20.1%), *Penicillium* spp. (19.8%), *Aureobasidium* spp. (18.4%), *Curvularia* spp. (16.2%), and *Nigrospora* spp. (15.3%). The prevalence of *Cladophialophora* has been reported in studies of aerosols from several Brazilian cities.[6,13]

During the study period, the hospital underwent extensive renovation for the implementation of a new unit, and this coincided with an increase in propagule counts, especially of *Cladophialophora* spp. In the period preceding renovation, there was a significant increase in *Fusarium* spp. fungi ($p<0.05$), which are potentially pathogenic and are currently being reported in cases of invasive disease.[20] *Aspergillus* spp. were isolated from the special areas in both periods, and, at similar levels to *Cladophialophora* spp., from inside the surgical center during the afternoon in the month of May, coinciding with renovation. In this period there was an increase in air contamination outside the building (data not shown), and a concomitant increase in the contamination of internal air. The same genera of fungi were recovered from both environments, suggesting an influence of external factors on internal contamination levels, as has been described in the literature.[2] There was also an increase in levels of *Cladophialophora* spp. in all internal collection points when compared to the previous months, and counts of this fungus were increased in the external environment. After the completion of the renovation process, there was a gradual decline, tending towards former levels, especially with respect to the *Cladophialophora* genus in the surgical center during the afternoon, indicating the importance of environmental monitoring to guide preventive measures. Several studies suggest that the distribution of fungi, both in terms of concentration and of the different genera encountered, vary among geographical areas and is influenced by environmental and seasonal factors.[6,11,13,17,24]

The levels encountered are considered as high for such environments, especially if compared to the levels reported by Távora et al.[24] A complicating factor is the lack of reference values for climate-controlled environments considered as 'special.' Mean levels in the collection points outside the ICUs and surgical center were higher than those found in internal environments, but lower than those found outside the building. The two sectors investigated were climate-controlled, but did not use HEPA filters. However, climate control in the ICUs was less regular, and natural ventilation was used on occasion. A difference in the total number of fungi isolated is evident in the surgical center, probably due to the different routines of the two sectors.

In a study lasting for over 30 weeks carried out during the partial renovation of a hospital in Pittsburgh, USA, Overberger et al[17] (1995) isolated *Penicillium* spp., *Aspergillus* spp., and, to a lesser extent, *Alternaria* spp., commonly isolated fungi, and *Cladosporium* spp. were the fungi most frequently isolated from the air. The authors reported an increase in levels of viable spores during renovation, and a subsequent decrease to former levels after renovation was concluded, results similar to those of the present study. Likewise, Bouza et al[2] (2002) found that the demolition of a hospital building was associated with increased colony counts in external and non-HEPA protected internal air, and that counts returned to former levels after 11 days. *Aspergillus fumigatus* was the fungus most frequently isolated, followed by *Penicillium* spp., *Aspergillus niger, Aspergillus flavus, Aspergillus* spp., and *Fusarium* spp., among other species. On the other hand, *Aspergillus* spp. were the filamentous fungus most frequently isolated by Panagopoulou et al[18] (2002) in a multicenter hospital study, corresponding to 70,5% of all isolates. Mousa et al[14] (1999), while investigating the correlation between fungi isolated from burn wounds and environmental surfaces of a burn care unit, found a greater proportion of *Aspergillus niger* in both patients and environments. However, *Ulocladium* spp. were the most common isolates in the control group (cultures obtained from outside the burn care unit), suggesting that patients may have acquired fungal infections while in the burn care unit.

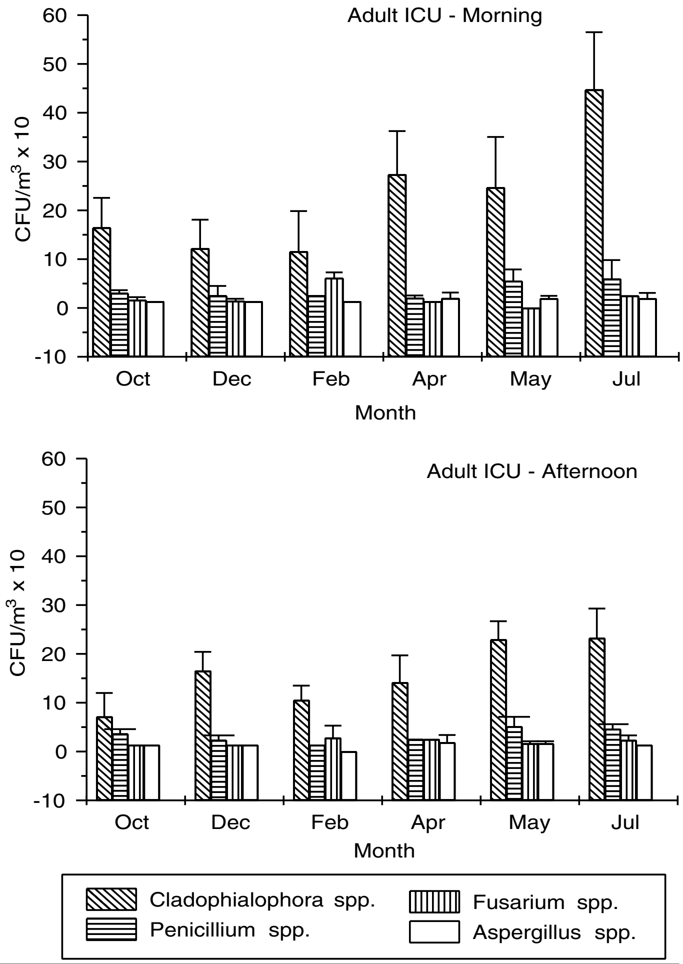

Figure 2 - Major genera isolated from the adult ICU, in CFU/m^3 (geometric mean ± standard deviation), during morning and afternoon collection periods in the months of October, December, February, April, May and July.

In the present study, we also monitored the area around the beds and the healthcare professionals that worked inside the surgical center and ICUs. *C. albicans*, described in the literature as the major agent of nosocomial infections,[4] was the prevailing species among the samples obtained from the healthcare staff. The presence of yeasts on the hands of these professionals is a source of concern for hospital-infection control teams, since this may be a potential source of contamination and dissemination of microorganisms. In the present survey, yeasts were found in samples obtained from 39.4% of the healthcare staff. Data on this subject in the literature show great disparity, with rates ranging between 17% and 80%.[8,23]

Candidiasis and invasive aspergillosis have increased in recent years, especially among immunocompromised patients.[1,25] *C. albicans* is still the major species involved in fungemia, although non-*albicans* species have increased in frequency, and are often refractory to conventional treatment.[3,4] On

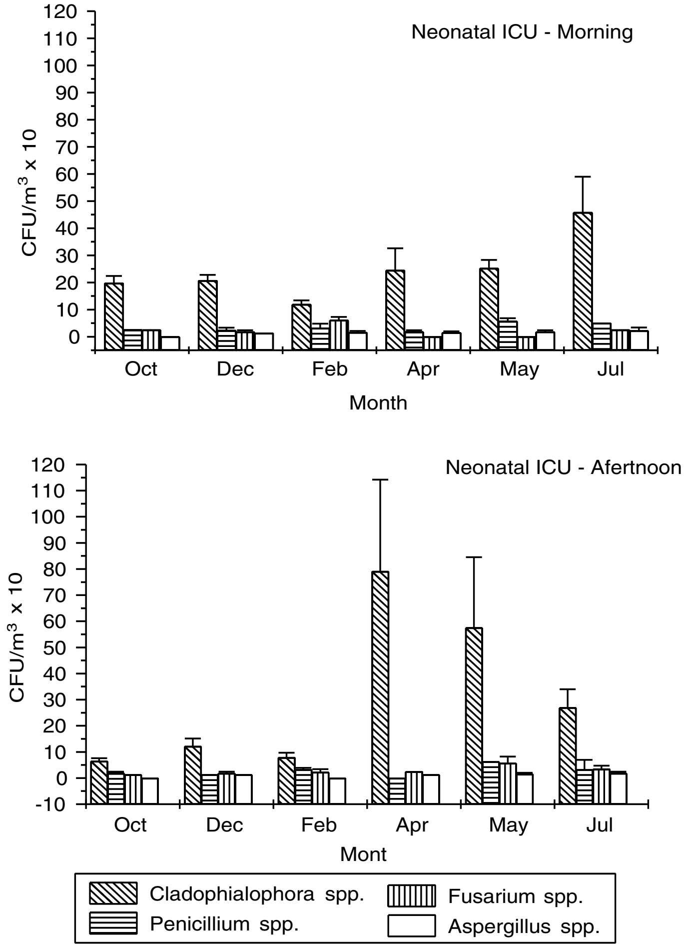

Figure 3 - Major genera isolated from the neonatal ICU, in CFU/m^3 (geometric mean ± standard deviation), during morning and afternoon collection periods in the months of October, December, February, April, May, and July.

the other hand, infections by resistant microorganisms are increasing, resulting in higher mortality rates, increased hospitalization periods, and consequent higher cost to hospital services.[21]

In the present study, yeasts of the *Trichosporon* genus, considered as emergent agents of hospital infections,[15] were also isolated at substantial levels, both from the healthcare staff and from all environmental surfaces investigated.

Of the samples obtained from the surfaces of furniture, two *Candida lusitaniae* isolates are worthy of note, since this yeast has mechanisms to develop resistance to Amphotericin B (certain strains being primarily resistant).[7] One of these samples was isolated from a doorknob in the neonatal ICU, and the other in the following week from a doorknob in the adult ICU, which are physically close to each other. Although a genotypic characterization was not carried out, such a result may suggest that yeasts are carried from one place to the next within critical areas. Although *Candida* spp. were the yeasts most frequently isolated from healthcare staff and environment, the isolation of *Trichosporon* spp. was important due to its potential to resist antifungal therapy.

The characterization of environmental fungi from internal environments in critical hospital areas and of the fungal microbiota found on the hands of healthcare professionals is recognized worldwide as an important measure aimed at substantially reducing morbidity, mortality, and high hospital costs.

Environmental sources must hence be monitored, especially in special rooms, which accommodate immunosuppressed patients, susceptible to environmental pathogens exposure. Only then, it will be possible to design appropriate measures for controlling such pathogens and to choose the adequate patients therapies to be instituted.

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