



Airborne fungi in an intensive care unit

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(With 1 figure)

Abstract

The presence of airborne fungi in Intensive Care Unit (ICUs) is associated with increased nosocomial infections. The aim of this study was the isolation and identification of airborne fungi presented in an ICU from the University Hospital of Pelotas – RS, with the attempt to know the place's environmental microbiota. 40 Petri plates with Sabouraud Dextrose Agar were exposed to an environment of an ICU, where samples were collected in strategic places during morning and afternoon periods for ten days. Seven fungi genera were identified: *Penicillium* spp. (15.18%), genus with the higher frequency, followed by *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., *Paecelomyces* spp., *Curvularia* spp., *Alternaria* spp., Zygomycetes and sterile mycelium. The most predominant fungi genus were *Aspergillus* spp. (13.92%) in the morning and *Cladosporium* spp. (13.92%) in the afternoon. Due to their involvement in different diseases, the identified fungi genera can be classified as potential pathogens of inpatients. These results reinforce the need of monitoring the environmental microorganisms with high frequency and efficiently in health institutions.

Keywords: hospital, ICU, air quality, infection.

Fungos anemófilos em uma unidade de terapia intensiva

Resumo

A presença de fungos anemófilos nas UTIs está associada com o aumento de infecções nosocomiais. O objetivo deste estudo foi isolar e identificar quais os principais fungos anemófilos presentes em uma Unidade de Terapia Intensiva (UTI) de um Hospital Universitário de Pelotas – RS, na tentativa de conhecer a microbiota ambiental do local. Através de 40 placas de Petri com Agar Sabouraud dextrose expostas no ambiente de UTI foram coletadas amostras por exposição em locais estratégicos durante períodos da manhã e tarde por dez dias. Sete gêneros fúngicos foram identificados: *Penicillium* spp. (15,18%), o gênero de maior frequência, seguido de *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., *Paecelomyces* spp., *Curvularia* spp., *Alternaria* spp., além de Zygomicetos e micélios estéreis. Houve predomínio de *Aspergillus* spp. (13,92%) pela manhã e *Cladosporium* spp. (13,92%) a tarde. Por estarem envolvidos em diferentes enfermidades, os gêneros identificados podem ser classificados como patógenos em potencial aos pacientes internados. Estes resultados reforçam a necessidade de um monitoramento dos micro-organismos ambientais com maior frequência e eficiência nas instituições de saúde.

Palavras-chave: hospital, UTI, qualidade do ar, infecção.

1. Introduction

Fungi are ubiquitous organisms widely distributed in the environment, it can be found in the plants, animals, soil, water and air (Carmo et al., 2007). These microorganisms are carried through water, insects, humans and animals, having the ability to disperse through the atmospheric air, being called airborne (Mezzari et al., 2003).

Invasive mould infections represent a threat for high-risk patients hospitalized in Intensive Care Unit (ICU). Hospital infections caused by fungi have been frequently reported in hospitalized patients, with a high morbidity and mortality, making it increasingly important to the awareness of air quality. It can be estimated that in Brazil,

the mortalities' rates referring to infections associated to the health care, reach 38.4% of the hospitalized patients (Souza et al., 2015), meanwhile, as reported worldwide, from each 100 hospitalized patients, seven acquired infection associated to health care in development countries and ten in under development countries (WHO, 2011).

Many hospital acquired infections are exogenously due to the increased use of air conditioning systems, which together with the accumulation of moisture are a great source of fungal spread (Martins-Diniz et al., 2005; Colombo, 2000; Pfaffer, 1996).

Although, fungal spores have the capacity to develop into different environments, they are considered inoffensive in immunocompetent subjects, but fatal in hospital inpatients receiving immunosuppressive medication (Afshari et al., 2013). It should be considered factors such as the fungal concentration, genus and species diversity, as well as their distribution characteristics in the environment, as shocking when considering the immunocompromised patients' condition (Zhang et al., 2015).

There are several reports in hospitalized patients affected by mycoses caused by airborne filamentous fungi present in the environment (Peckham et al., 2016; Caggiano et al., 2014; Shams-Ghahfarokhi et al., 2014), meanwhile in Brazil, few studies (Flores and Onofre, 2010; Lobato et al., 2009; Carmo et al., 2007; Martins-Diniz et al., 2005) characterizing the airborne fungi microbiota in the ICUs' environment, being an important knowledge for control strategies. The aim of this study was the isolation and identification of airborne fungi in an ICU from the University Hospital of Pelotas (RS – Brazil).

2. Material and Methods

The survey of the airborne fungi in the ICU was performed at the University Hospital of Pelotas, RS, during the months from September to November. For the analysis, ten samples were collected through the Sedimentation Plate Method using Sabouraud Dextrose Agar Medium. For collection, eight dishes were exposed each day, for a

20 minutes period. Dishes were located under three room air-conditioners, having one air-conditioner in each room and also located where hospital materials are disposed, at approximately one meter height above ground (Flores and Onofre, 2010; Carmo et al., 2007).

For each day of the week, four samples from the morning period and four samples from the afternoon period were collected, totaling 40 plates during the ten days of study (20 plates in the morning and 20 plates in the afternoon). Two samples were collected in one day from a period of five days, in other words, there were collection of samples in the morning and afternoon shifts, in the same day of the week, and each day corresponded with a different day of the week. Two sampling collection periods were performed, because general cleaning and sanitation of the place was performed in one hour before taking the samples in the same two studied periods.

After the plates were collected, they were incubated at 25 °C for seven days. The identification was performed through visualization of the macro and micromorphology characteristics of the growing colonies and the production of blades with visualization Lactophenol cotton blue and slide culture between blades when needed (Sidrim and Rocha, 2004).

3. Results

From the 40 collected plates exposed into the ICU's environment, 33 plates (82.5%) showed growth of 79 filamentous fungi colonies. The filamentous genera were established in the Figure 1.

From the 20 collected plates on the morning period, 15 (75%) presented fungal growth and from the 20 plates of the afternoon period, 18 (90%) showed fungal growth. Higher fungal isolation was shown on the afternoon period.

Seven fungal genera were identified (Figure 1) being: *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., *Paecilomyces* spp., *Curvularia* spp., *Alternaria* spp. and by Zygomycetes and sterile mycelium.

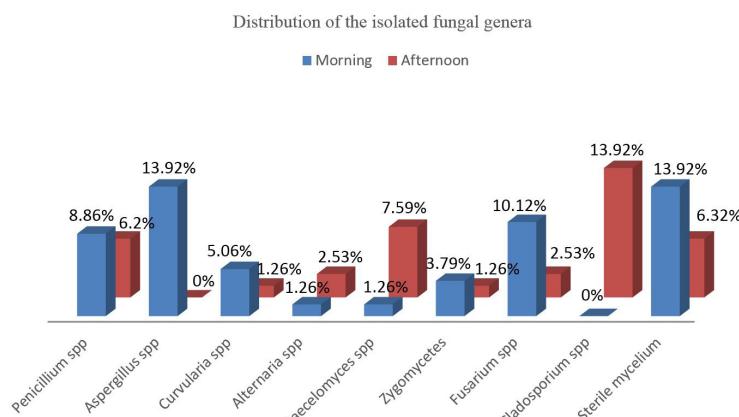


Figure 1. Distribution of the isolated fungal genera from the Intensive Care Unit at the Pelotas University Hospital according to the time from collection.

Aspergillus spp. (13.92%) was the most predominant fungi genus in the morning period and the *Cladosporium* spp. genus (13.92%) in the afternoon period, both were identified in one of the two evaluated shifts.

4. Discussion

For many years hospital fungal isolation has been reported such is the case of *Aspergillus* spp., *Penicillium* spp., *Cladophialophora* spp. and *Fusarium* spp. yet related with the Unit construction and renovation, providing conditions for the appearance of this kind of microorganisms in such places (El-Sharkawy and Noweir, 2014; Sautour et al., 2007; Martins-Diniz et al., 2005; Iwen et al., 1994).

Considered potentially pathogenic, the occurrence of the genera *Penicillium*, *Aspergillus* and *Cladosporium*, which stood out in this study, are reported as predominant in the hospital environment by others authors (Melo et al., 2009; Martins-Diniz et al., 2005). The fact that *Aspergillus* spp. and *Cladosporium* spp., were isolated only in one of the shifts is probably due, by the different hospital's staff and routines in each period and by the abiotic conditions in which this experiment was done (Melo et al., 2009; Bernardi and Nascimento, 2005; Mezzari et al., 2003).

Despite the cleaning and sanitizing procedures realized according to the normative instructions, 82.5% of the isolates using Petri plates is a high rate to be observed in a hospital unit, mainly in an ICU. During the isolates' perform, the genus *Penicillium* was the most isolated fungi, meanwhile, a moderate frequency in the morning (8.86%) and in the afternoon (6.32%) periods was observed. Even so its presence becomes relevant by being involved in reported cases of disseminated infections (Ye et al., 2015), multiple brain abscesses (Noritomi et al., 2005), peritonitis (Böhlke et al., 2007) and pneumonia in immunocompromised patients (Oshikata et al., 2013).

In Brazil, similar studies have been conducted at various locations, including Campina Grande (PB), Francisco Beltrão (PR), Rio Grande (RS) and Pouso Alegre (MG). In these cities, the most frequently isolated genus was *Penicillium* spp. varying in frequency from 40% to 93.3% (Flores and Onofre, 2010; Souza Júnior and Vieira, 2010; Melo et al., 2009; Lobato et al., 2009; Carmo et al., 2007). These frequency results were higher than those observed in this study, however according that *Penicillium* spp. was also the most frequently isolated genera, can be associated with the different atmospheric conditions and immunologic status of the patients in the execution period of the different researches.

Regarding that *Aspergillus*, is a fungus genus with a worldwide distribution, the most common species include *A. flavus* and *A. fumigatus* (Lacaz et al., 2002). Aspergillosis is related with different syndromes that can be manifest as allergic reactions or as invasive forms, otomycosis, endocarditis, osteomyelitis and skin infections, among others (Wanke et al., 2000). The genus *Aspergillus* spp., was reported by Panagopoulou et al. (2002) with a higher frequency than the observed in this study. When

evaluating three hospitals in Greece for the presence of airborne fungi, the authors recorded the presence of this genus in 70.55% of their samples.

Lugauskas and Krikštonis (2004) investigated the diversity of airborne fungi at hospitals in Lithuania and made the isolation of 41 genera, including *Penicillium* spp., as the most predominated species, followed by *Aspergillus* spp., *Acremonium* spp., *Paecilomyces* spp., *Mucor* spp. and *Cladosporium* spp. Their results were similar to those found in our study with the exception of the genera *Acremonium* spp. and *Mucor* spp.

Cladosporium spp. is a dematiaceous fungus, that contains melanin in their cellular walls, which brings them a dark color in their spores and hyphae, associated most of the time to the microorganism's virulence (Revankar, 2007), causes mainly infections of the central nervous system such as brain abscess between others (Freitas et al., 1997; Goel et al., 1992). Mainly in relation to the genus *Cladosporium*, its frequency it's also elevated in relation to the hospital environment as observed by Diongue et al. (2015) who obtained a percentage of 91.1% of all isolates related to this genus in a hospital ward. Apparently, *Cladosporium* spp. is associated with damages in the respiratory function, as demonstrated by Chen et al. (2014) associating the children exposition to the fungi having a reduction in the pulmonary function. These findings are higher in children with asthma (Vicendese et al., 2015).

In the same way, cases of infections caused by these environmental fungi had been described in immunocompetent individuals in locals with presence of high quantity of fungal inoculums. For example, related mycoses such as: disseminated pediculosis (Ye et al., 2015), mycotic endophthalmitis (Wu et al., 2011), intracranial aspergiloma (Mohammadi et al., 2015), cerebral aspergillosis (Bokhari et al., 2014), pulmonary scedosporium (Rahman et al., 2016), gastrointestinal aspergillosis (Koutsounas and Pyleris, 2015) between others diagnosis found in literature (Zahid and Farooqi, 2015; Borkar et al., 2008). This problem aggravates in immunocompromised patients, such is the case of hospitalized children with hypersensitivity related to the presence of the fungi inside the hospital (Beck et al., 2015; Libbrecht et al., 2015; Guppy et al., 1998).

It is worth mentioning that, also fungi with low prevalence must be considered relevant. This is the case of the Zygomycetes fungi which occurred in this study in a low frequency (5.05%), responsible of the zygomycosis which is a rare infection, however invasive and with fast evolution, affecting immunosuppressed and eventually immunocompetent subjects (Souza et al., 2014; Severo et al., 2010; Prabhu and Patel, 2004).

Allergic reactions in humans may be by the inhalation of airborne fungi, this determines the intensity of clinical significance, being able to develop respiratory diseases, among other diseases (Mezzari et al., 2003). Due to their invasive and opportunistic agents the presence of the genera *Penicillium* spp. and *Aspergillus* spp. in hospitals can cause high morbidity and mortality in immunocompromised patients, such as cancer patients in chemotherapy treatments,

AIDS patients, patients with multiple trauma and among others are the most affected by opportunistic mycoses (Vesper et al., 2007; Moretti, 2007; Pfaffer, 1996).

Economic loss is another aspect to be considered in the nosocomial infections. According to the disclosed data by the World Health Organization (WHO, 2011), only in the state of Minas Gerais, Brazil, the expenses in the year 1992 with infections related to health care were of US\$ 18 million. Public institutions are attributed to have the highest rates of nosocomial infections, providing an increase in the costs of treatment three times higher than the subjects with not acquire infection, with episodes that are concentrated in the ICUs due to the presence of subjects with a higher severity clinical case and by their time of permanence in the ICU area (Moura et al., 2007; Prade, 1995).

Studies conducted by Shams-Ghahfarokhi et al. (2014), Caggiano et al. (2014) and Chang et al. (2014) confirmed that the environmental monitoring of airborne filamentous fungi is necessary to reduce fungal concentrations in operating theaters and in controlled environments, to prevent infections. According to Chang et al. (2014), in the tentative to reduce the environmental contamination by fungi, recommended that a standardized protocol for the sampling collection and culture is necessary in hospital institutions to verify the air quality.

5. Conclusion

In conclusion, the isolates in the ICU at the University Hospital from Pelotas were *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., *Paecelomyces* spp., *Curvularia* spp., *Alternaria* spp., Zygomycetes and sterile mycelium. The presence of these pathogens emphasizes the importance of microbial control measures in the ICU.

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