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ARTIGO ORIGINAL

Seleção dos fungos anemófilos *Acremonium* sp. e *Fusarium* sp. em uma central hospitalar de diluição de medicamentos depois do procedimento de limpeza com hipoclorito

Selection of the anemophilous fungi Acremonium sp. and Fusarium sp. in a hospital drug dilution center after the cleaning procedure with hypochlorite

Selección de hongos aerotransportados Acremonium sp. y Fusarium sp. en un centro de dilución de medicamentos del hospital después del procedimiento de limpieza con hipoclorito

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RESUMO

As infecções oportunistas têm recebido mais atenção nos últimos anos devido ao grande número de pessoas imunologicamente suscetíveis expostas. Em um hospital, onde se concentram pacientes suscetíveis, os fungos podem ser considerados um problema por apresentarem estrutura resistente e de fácil disseminação. O processo de limpeza periódica das superfícies e o monitoramento da qualidade do ar constituem estratégias importantes para a prevenção de infecções. Nesse contexto, o objetivo deste estudo foi avaliar a presença de fungos anemófilos no ar de uma central hospitalar de diluição de medicamentos, antes e após o procedimento de limpeza semanal com 1% de hipoclorito. As análises quantitativas e qualitativas foram realizadas expondo placas de Petri com ágar Sabouraud de acordo com o esquema 1/1/1 (por 1 hora, 1 m acima do chão e cerca de 1 m de distância das paredes ou de outra placa). Após a incubação por 5 dias a 25 ° C, as UFC foram quantificadas, isoladas e incubadas novamente para análises macroscópicas e microscópicas. A amostragem mostrou uma contaminação de 57 CFU (14 CFU / placa em média) e 50 CFU (12 CFU / placa em média) antes e depois do procedimento de limpeza, respectivamente. Os fungos encontrados antes da limpeza foram *Aspergillus* sp., *Penicillium* sp., *Curvularia* sp., *Acremonium* sp. e *Fusarium* sp., enquanto após a limpeza *Acremonium* sp. e *Fusarium* sp.. Este estudo revelou que a qualidade do ar da central de diluição estava comprometida mesmo após o processo de limpeza e a eficiência do processo de limpeza, bem como, os produtos utilizados deveriam ser avaliados constantemente para garantir a eficácia do procedimento .

Descritores: *fungos anemófilos, Fusarium sp., limpeza hospitalar, hipoclorito.*

ABSTRACT

Opportunistic infections have been received more attention in the last years since the large number of people immunologically susceptible exposed. In a hospital, where susceptible patients are concentrated, fungi may be considered a problem

because they have natural resistant structure and easy dissemination. Periodic cleaning process of surfaces and monitoring of the air quality comprise important strategies to preventing infections. In this context, the aim of this study was to evaluate the presence of anemophilous fungi in the air of a hospital drug dilution center, before and after the cleaning weekly procedure with 1% of hypochlorite. Quantitative and qualitative analyses were performed by exposing Petri dishes with Sabouraud agar according to the 1/1/1 scheme (for 1 hour, 1 m above the floor and about 1 m away from walls or another dish). After incubation for 5 days at 25 °C, CFU were quantified, isolated and incubated again for macroscopic and microscopic analyses. Sampling has shown a contamination of 57 CFU (14 CFU/plate on average) and 50 CFU (12 CFU/plate on average) before and after the cleaning procedure, respectively. The fungi found before cleaning were *Aspergillus* sp., *Penicillium* sp., *Curvularia* sp., *Acremonium* sp. and *Fusarium* sp., whereas after cleaning were *Acremonium* sp. and *Fusarium* sp.. This study revealed that the air quality of the drug dilution center was compromised even after the cleaning process and the efficiency of the cleaning process, as well as, the products used should be constantly evaluated to ensure the effectiveness of the procedure.

Key words: *anemophilous fungi, Fusarium sp., hospital cleaning procedure, hypochlorite.*

RESUMEN

Las infecciones oportunistas han recibido más atención en los últimos años debido a la gran cantidad de personas inmunológicamente susceptibles expuestas. En un hospital, donde se concentran pacientes susceptibles, los hongos pueden considerarse un problema porque tienen una estructura resistente y son fáciles de propagar. El proceso de limpieza periódica de superficies y el monitoreo de la calidad del aire son estrategias importantes para prevenir infecciones. En este contexto, el objetivo de este estudio fue evaluar la presencia de hongos aerotransportados en el aire de un centro hospitalario de dilución de medicamentos, antes y después del procedimiento de limpieza semanal con hipoclorito al 1%. Los análisis cuantitativos y cualitativos se realizaron exponiendo cajas de Petri con agar Sabouraud según el esquema 1/1/1 (durante 1 hora, 1 m sobre el piso y aproximadamente 1 m de distancia de las paredes u otra placa). Después de una incubación de 5 días a 25 °C, las UFC se cuantificaron, aislaron y volvieron a incubar para análisis macroscópicos y microscópicos. El muestreo mostró una contaminación de 57 CFU (14 CFU/placa en promedio) y 50 CFU (12 CFU/placa en promedio) antes y después del procedimiento de limpieza, respectivamente. Los hongos encontrados antes de la limpieza fueron *Aspergillus* sp., *Penicillium* sp., *Curvularia* sp., *Acremonium* sp. y *Fusarium* sp., mientras que después de limpiar *Acremonium* sp. y *Fusarium* sp.. Este estudio reveló que la calidad del aire de la planta de dilución se vio comprometida incluso después del proceso de limpieza y la eficiencia del proceso de limpieza, así como los productos utilizados, deben evaluarse constantemente para garantizar la eficacia del procedimiento.

Palabras clave: *hongos aerotransportados, Fusarium sp., limpieza hospitalaria, hipoclorito.*

INTRODUCTION

Hospital infection is a serious public health problem and can exhibit great complexity, severity, and economic and social implications. Hospital infection has increased the rates of morbidity, mortality, costs and inpatient time-hospitalization. Among the threatening sources of microorganisms are the hospital's internal surfaces, the air, the refrigeration system and the health staff, that carry the microorganisms. These microorganisms become a risk for inpatients that are more likely immunocompromised, weakened and susceptible due to the existing pathology (Sydnor and Perl, 2011).

Airborne transmission occurs by microorganisms carried by droplets, dust or particles suspended in the air that can reach distant places from the primary source, what can usually make difficult to find the origin of the contamination. These microorganisms can be inhaled or deposited on inappropriate surfaces (Sydnor and Perl, 2011; Napoli *et al*., 2012). Fungi are example of microorganisms that use this route of transmission. In addition to easy dissemination, fungi are able to grow in almost all natural and synthetic materials and exhibit some resistance to chemical destruction due to the spore formation ability (Khan and Karuppayil, 2012).

In general, in the last decades, an increase in hospital infection caused by fungi has been observed (Sydnor and Perl, 2011; Alangaden, 2011). Studies have shown that *Candida* spp. is the main yeast related to hospital infection. But the most common anemophilous-filamentous fungi genus is *Aspergillus* spp.. However, other fungi have also been implicated in nosocomial infections, including: *Penicillium* spp., *Fusarium* spp., *Scedosporium* spp., *Paecilomyces* spp., *Phialemonium* spp., *Curvularia* spp. and *Pneumocystis* spp. (Alangaden, 2011).

Besides mycosis, fungi can be responsible for allergy, bronchial irritation, hypersensitivity syndromes or serious systemic infections, especially in immunocompromised people. Chronic obstructive pulmonary disease, asthma and cystic fibrosis are pathologies in which complications with *Aspergillus* are common (Baxter *et al*., 2011). In patients with asthma and cystic fibrosis, *Aspergilus spp*. can cause bronchopulmonary aspergillosis, semi-invasive and invasive pulmonary aspergillosis, and pulmonary aspergilloma (Kawel *et al*., 2011). Pisa *et al*., 2015 also associated Alzeimer's disease to fungal infection, due to the angiopathy and hyphae found in patients' brain tissue and the deposition of amyloid substance in the neurovascular system.

Controlling the risk factors involved in the fungi transmission and monitoring the limitation offered by the disinfection and cleaning methods is possible to reduce the hospital infection. Surgical, onco-hematological and drug dilution rooms are places that must constantly undergo cleaning process for microorganisms reduction or elimination. Thus, it is expected that the cleaning process should promote efficient removal of the microorganisms through proper products and procedures used. For that, the air quality monitoring should be also be performed periodically.

Thus, as the air is one of the most important vehicle for microorganisms dissemination in a hospital, mainly for fungi, and the Drug Dilution Center (DDC) room is a place that must have minimum or absence of contamination due to the drug manipulation, the aim of this study was to evaluate the

presence of anemophilous fungi in the air of a hospital drug dilution center located in Rio Grande do Sul state, Southern Brazil, before and after the cleaning weekly procedure with 1% hypochlorite.

METHODS

The airborne fungi in a Drug Dilution Center (DDC) of a medium-size hospital with 165 beds, located in the south of Brazil, were evaluated by passive sampling. Four Petri dishes (9 cm of diameter) with Sabouraud agar (ACUMEDIA) were exposed according to the 1/1/1 scheme: for 1 hour, 1 m above the floor and about 1 m away from walls and another dish (Pasquarella *et al*., 2000). Two passive sampling were performed, one before and another after the cleaning weekly process with 1% hypochlorite. One sampling happened twelve hours after the terminal-weekly cleaning practice and the other prior to the weekly cleaning process (six days after the last cleaning procedure). After the exposition, plates were incubated at 25 °C for 5 days for CFU quantification.

Subsequently to the quantification, colonies were picked separately and incubated at 25 °C and 37 °C for 5-7 days for macroscopy analysis (Falvey and Streifel, 2007). Genus confirmation was carried out by microscopic morphology analyses of the fungus through the microculture, according to the literature (Winn, 2001; Minami, 2003; Maza *et al*., 1999).

Terminal cleaning procedure in the DDC and anteroom is usually performed once a week, usually on Saturday. This cleaning procedure is carried out with hypochlorite 1% and covers the floor, walls and countertops. In the other days of the week the cleaning of the benches is done at the end of each team shift, three times a day, using alcohol 70%. The DDC has twenty professionals and operates 24 hours. Employees use the following IPE (individual protection equipment): local coat, hat, gloves and mask. The average time that the prepared drugs remain in the Central is two hours.

RESULTS

Airborne fungi evaluation showed a contamination of 57 CFU (14 CFU/plate on average) of fungi before cleaning and 50 CFU (12 CFU/plate on average) after cleaning procedure with 1% hypochlorite.

Aspergillus sp., *Penicillium* sp., *Curvularia* sp., *Acremonium* sp. and *Fusarium* sp. were the fungi found before cleaning the DDC room. *Acremonium* sp., and *Fusarium* sp. were the fungi found after the cleaning procedure.

DISCUSSION

Monitoring and controlling anemophilous fungi contamination in hospital critical internal areas have been emphasized as a strategy to manage the nosocomial infection (Caggiano *et al*., 2014). The DDC consists of one of these areas since in this place oral and injectable medicines are prepared and reach immunocompromised patients, patients hospitalized for a long time and patients with serious pathologies. For these reasons, the cleaning of this place should be frequent and efficient. In this context, we verified the level and type of anemophilous fungi contamination in a hospital DDC, as well as, we analyzed the efficiency of the cleaning process.

The cleaning process has provided a decrease of approximately 12% of the total colony counts, disappearing the genera *Aspergillus* sp., *Penicillium* sp. and *Curvularia* sp.. This reduction, however, was lower than expected, since there was a selection of resistant genera, *Acremonium* sp. and *Fusarium* sp., that proliferated and appeared in greater number than in the previous testing.

In this case, the product used and/or the procedure applied were not efficient for cleaning the area, since they did not reduce the contamination drastically and, in addition, have selected resistant strains. The result of this study shows the importance of evaluating the cleaning process, as well as, and assessing the concentration and quality of the disinfectant used for the procedure.

Another important fact that concerns is related to the type of fungi found, since the presence of them in the DDC suggests the presence of them in other hospital's rooms. Fungi like *Aspergillus* sp, *Penicillium* sp. and *Curvularia* sp. are associated to pathologies mainly in immunocompromised patients. *Penicillium* sp. and *Aspergillus* sp. are usually the main genera found in hospital settings and other internal environments. Khan and Karuppayil (2012) have showed that such genera appear in more than 90% of the 150 environments surveyed in different continents.

Aspergillus species represent the second cause of fungal infections, behind only to *Candida* sp. An invasive aspergillosis incidence is estimated of 5 cases per 100,000 inhabitants, with a mortality rate between 45-80%. *Aspergillus* is ubiquitous and the spores are easily inhaled by dust from sites under construction or by water droplets from the cooling system. Water system and non-use of HEPA filters have been incriminated as responsible for fungi spores dissemination. Aspergillosis has been seen most often in individuals with blood disease, transplanted patient or those receiving high doses of steroid hormones. However, this genus has also been isolated from burn wounds. Aspergillosis frequency is higher 2 to 3 weeks after transplantation. Immunocompromised people should avoid segments of hospitals that are under construction (CDC 2007, Sydnor and Perl, 2011).

Infections by *Fusarium*, called fusariosis, have been considered less common than by *Candida* and *Aspergillus*, and they usually happen in people severely immunocompromised (Sydnor and Perl, 2011). Fusariosis can be localized or become disseminated and have been observed affecting the cornea (keratomycosis, endopthalmitis), but it has also been implicated in onychomycosis, catheter infections, peritonitis, sinusitis, septic arthritis and serious nosocomial infections (Nucci and Anaissie, 2007; Chowdhary *et al*., 2014). *Curvularia* spp., despite very rare, was already isolated from saline-filled breast implants (Alangaden, 2011).

Khan and Karuppayil (2012) consider the best way to ensure air quality by using filters in critical hospital environments or keeping relative air humidity below 50%. However, the Centers for Disease Control and Prevention adopt the following aspects for the air quality maintenance: control of the temperature between 22º C to 24º C, air movement from clean to the less clean areas, and air intake should be at the top and exhaust in the lower part of the room by positive pressure, with 15 total air changes per hour. The recirculation should occur through filters. In addition, the CDC's guideline indicates the chlorine dilution in ppm (parts per million) that should be calculated taking into account the concentration of the original product, which changes depending on the brand.

We have showed here that in addition to performing terminal cleaning continuously the critical hospital environments, it is necessary to evaluate the efficiency of this process. Further, the chlorine dilution CDC's guideline recommendation in ppm (parts per million) should be taken in consideration to guarantee the suitable chlorine concentration. In addition,

with the emergence of resistant microorganisms monitoring our environment and be aware of the threats that we will have to face in the future is prevention.

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