Evaluation of total fungal air contamination levels and efficiency of the ventilation systems used in adult haematology unit and adult stem cell transplantation unit

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This study is aimed to measure the fungal loads in the protected areas with different ventilation systems, including the stem cell transplantation unit and haematology service to compare the results with the outdoor air. Using volumetric air sampling method, fungal load was investigated in the air; samples were taken from 11 points for a period of 25 weeks. Concentrations of fungal conidium were identified by the sampling carried out in the stem cell transplantation unit patient rooms, stem cell transplantation unit corridor and entrance area, adult haematology service patient rooms, haematology service corridor and hospital garden. Considering the total fungi load in all groups; Fonsecaea sp. (40.6%), Penicillium sp. (32.9%), Alternaria sp. (7.1%), Aspergillus sp. (5.8%) and Arthrographis sp. (3.3%) were found to be the most common species. In this study, effect of the ventilation system on the total fungus concentration was obviously seen. But, there was no obvious difference in terms of the Aspergillus concentrations between high-efficiency particulate air (HEPA) filtered stem cell transplantation unit and haematology service where a filter of lower efficiency was used. There was not an obvious difference between the indoor and outdoor locations in terms of the distribution of identified species. This might be explained with outdoor fungi contamination reflecting to the indoor location in a certain amount.

Key words: Haematology, stem cell transplantation, air contamination, fungi, Aspergillus sp.

INTRODUCTION

Advances in modern medicine have brought a significant increase in the number of patients under the high risk for opportunistic infections with a suppressed immune system. Among these opportunistic infections, nosocomial fungal infections take an important place with high mortality rate and treatment cost (Haiduven et al., 2009). Aspergillus species that we encounter more as pulmonary infections are the most common opportunistic mold fungi (Kousha et al., 2011).

Even with intensive medication and antifungal treatment, invasive Aspergillosis (IA) has become a leading cause of death for patients with haematologic
malignancies and bone marrow transplantations. IA is known to be an airborne infection. Although a quantifiable level of contamination leading to an increased risk of infection has not been determined, increased airborne Aspergillus spp. have been correlated with increased incidence of IA (Alberti et al., 2001; Arnow et al., 1991; VandenBergh et al., 1999).

The patients with suppressed immune systems who followed-up in haematology service (HS) and stem cell transplantation unit (SCTU) where nosocomial fungal infections pose the highest rate of problems are under a great risk due to particles carrying microorganisms (VandenBergh et al., 1999; Leenders et al., 1999). Given difficulties in the diagnosis and poor treatment results, increasing the air quality in the hospital and keeping the concentrations of Aspergillus conidia under control seems an effective strategy in order to prevent IA infections (VandenBergh et al., 1999; Erol, 2010; Fridkin and Jarvis, 1996; Perrioth et al., 2007; Richardson et al., 2000).

This study is aimed at determining for the first time the fungal contamination quantities in adult HS and SCTU of our hospital where the patients were under risk for invasive fungal infections. A further objective of this study was to investigate efficiency of the ventilation systems used in these critical locations of our hospital. For this purpose, air samples were collected from these parts of our hospital for a period of 25 weeks and the mean contamination values were obtained.

MATERIALS AND METHODS

Air samples were collected from total 11 areas of Akdeniz University Medical Faculty, including adult HS corridor, four patient room of HS service, two adult SCTU patient rooms, adult SCTU corridor and the corridor open to visitors in front of the SCTU (to represent the ventilation in other parts of the hospital), and two samples were collected from the hospital garden (to represent outdoor air) once a week for a period of 25 weeks from January to June.

The ventilation was provided by high-efficiency particulate air (HEPA) filters which were filtering the particles of 0.5 μm in size with an efficiency of 99.9% in the adult SCTU rooms, a filter in the adult HS which was filtering the particles of 0.5 μm in size with an efficiency of 95.9% and the fan system in the corridor in front of the SCTU unit with 35 to 40% efficiency similar to the other areas of the hospital. Depending on having similar ventilation systems, similar patient concentration of 11 study areas were divided into 6 groups (Table 1). The sampling operations were performed from the same points and at same time of the day (08:00 to 10:00 AM). Air sampling was performed using the air quality control device (airIDEAL BioMérieux, France) providing automated cultivation of a certain volume of air (with the vacuum effect in an air flow rate of 100 L/min) to the medium of Sabouraud dextrose agar (SDA) (Becton Dickinson, USA) containing gentamicin and chloramphenicol. Sampling SDA mediums were controlled daily at 25°C and incubated for 7 days.

Fungus colonies were counted, volume of the air was calculated depending on the sampling period (100 L per 1 min) and the corresponding number on the charts of the air quality control device was found and, the levels of contamination were calculated as CFU/m³.

Furthermore, macroscopic appearance of the cultivated fungi colonies in the medium was analyzed and the microscopic properties were evaluated by obtaining the pure cultures and staining with lactophenol cotton blue (Remel, USA). Statistical analyses were carried out using Mann Whitney U test and windows SPSS 10.0 statistical package software.

RESULTS

Considering the total fungi loads in all groups; Cladosporium sp. (40.6%) Penicillium sp. (32.9%), Alternaria sp. (7.1%). Aspergillus sp. (5.8%) were found to be the most common species (Table 1). The differences between the total fungal concentrations of the SCTU rooms and the other locations were found statistically significant (p<0.005).

Fungal concentrations collected from all the study areas are shown in Table 1. Mean concentrations of Aspergillus species collected from all the study areas are shown in Table 2. Considering the total fungi loads in all groups; 23.3% were found as A. fumigatus. 26.6% A. flavus, 25.3% A. niger and 35.8% were found as the other Aspergillus species (Table 2). The differences between Aspergillus sp. Concentrations of SCTU entrance area and all other areas were found to be significant (p<0.05).

DISCUSSION

Looking to the overall distribution identified in this study, Cladosporium sp., Penicillium sp., Alternaria sp. and Aspergillus sp. were the first five most common species. In addition, there was not an obvious difference between the indoor and outdoor locations in terms of the identified species and their distribution. This might be explained with outdoor fungi contamination reflecting to the indoor location in a certain amount.

There is no air contamination standard which is recognized worldwide for the critical parts of hospitals (Panagopoulou et al., 2002). However, previous studies noted the fungus contamination between 0.01 and 50 CFU/m³ depending on the conditions such as whether the study location was in a protected area, air filtration system and age of the building (Munoz et al., 2000). When the data of the total fungus concentration per cubic meter were assessed, effect of the ventilation system on the fungus contamination was obviously seen. In the measurements carried out, areas with the lowest fungal contamination in the air were the locations in SCTU which was ventilated with HEPA filtering system. These locations were followed by HS areas having one filter with 95.90% efficiency and the corridor in front of SCTU which was ventilated through a filter with 35 to 40% efficiency.

In their study, Pini et al. (2004) followed-up two haema-
Table 1. Distribution of the fungus spp. in the collected samples.

<table>
<thead>
<tr>
<th>Sampling area</th>
<th>Number of samples</th>
<th>Total fungi CFU/m³ mean-(range)</th>
<th>Aspergillus sp. CFU/m³ mean-(range)</th>
<th>Alternaria sp CFU/m³ mean-(range)</th>
<th>Arthrographis sp. CFU/m³ mean-(range)</th>
<th>Bipolaris sp. CFU/m³ mean-(range)</th>
<th>Cunninghamella sp. CFU/m³ mean-(range)</th>
<th>Fonsecaea sp. CFU/m³ mean-(range)</th>
<th>Cladosporium sp. CFU/m³ mean-(range)</th>
<th>Penicillium sp. CFU/m³ mean-(range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCTU</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient Rooms</td>
<td>50</td>
<td>4.18 (0-34)*</td>
<td>0.42 (0-3)</td>
<td>0.30 (0-3)</td>
<td>0.00</td>
<td>0.20 (10-10)</td>
<td>0.00</td>
<td>1.34 (0-17)</td>
<td>1.48 (0-13)</td>
<td></td>
</tr>
<tr>
<td>Corridor</td>
<td>25</td>
<td>14.44 (0-61)</td>
<td>1.52 (10-10)</td>
<td>1.44 (0-20)</td>
<td>1.00</td>
<td>0.00</td>
<td>0.40 (10-10)</td>
<td>5.44 (0-24)</td>
<td>2.96 (0-20)</td>
<td></td>
</tr>
<tr>
<td>Entrance Area</td>
<td>25</td>
<td>94.40 (7-741)</td>
<td>10.80 (0-51)</td>
<td>13.08 (0-200)</td>
<td>1.60 (0-20)</td>
<td>0.00</td>
<td>0.00</td>
<td>37.52 (0-186)</td>
<td>51.68 (0-500)</td>
<td></td>
</tr>
<tr>
<td>HS</td>
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<td></td>
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</tr>
<tr>
<td>Patient Rooms</td>
<td>100</td>
<td>63.48 (0-797)</td>
<td>3.17 (0-35)</td>
<td>2.60 (0-30)</td>
<td>1.79 (0-46)</td>
<td>0.45 (0-30)</td>
<td>0.05 (0-5)</td>
<td>0.00</td>
<td>25.64 (0-549)</td>
<td>26.57 (0-797)</td>
</tr>
<tr>
<td>Corridor</td>
<td>25</td>
<td>63.84 (0-186)</td>
<td>3.36 (0-20)</td>
<td>2.00 (0-10)</td>
<td>3.20 (0-30)</td>
<td>0.00</td>
<td>0.40 (10-10)</td>
<td>0.00</td>
<td>27.24 (0-175)</td>
<td>21.04 (0-109)</td>
</tr>
<tr>
<td>Hospital Garden</td>
<td>50</td>
<td>146.37 (0-553)</td>
<td>3.47 (0-50)</td>
<td>21.49 (0-123)</td>
<td>2.06 (0-61)</td>
<td>0.82 (0-20)</td>
<td>0.00</td>
<td>0.85 (0-22)</td>
<td>83.18 (0-653)</td>
<td>25.41 (0-318)</td>
</tr>
</tbody>
</table>

*(p<0.005)

Table 2. Distribution of the Aspergillus sp. in the collected samples.

<table>
<thead>
<tr>
<th>Sampling area</th>
<th>Number of samples</th>
<th>Total Aspergillus sp. CFU/m³</th>
<th>Percentage of samples positive for Aspergillus spp.</th>
<th>A.fumigatus CFU/m³ Mean (Range)</th>
<th>Samples positive for A. fumigatus (% of total Aspergillus recovered)</th>
<th>A.flavus CFU/m³ Mean (Range)</th>
<th>Samples positive for A. flavus (% of total Aspergillus recovered)</th>
<th>A. niger CFU/m³ Mean (Range)</th>
<th>Samples positive for A. niger (% of total Aspergillus recovered)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KHTU</td>
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<td></td>
</tr>
<tr>
<td>Patient rooms</td>
<td>50</td>
<td>0.42 (0-3)</td>
<td>14 (7/50)</td>
<td>0.12 (0-3)</td>
<td>28.5 (2/7)</td>
<td>0.00 (0-0)</td>
<td>0</td>
<td>0.12 (0-3)</td>
<td>28.5 (2/7)</td>
</tr>
<tr>
<td>Corridor</td>
<td>25</td>
<td>1.52 (0-10)</td>
<td>32 (8/25)</td>
<td>0.00 (0-0)</td>
<td>0</td>
<td>0.24 (0-3)</td>
<td>25 (2/8)</td>
<td>0.88 (0-10)</td>
<td>62.5 (5/8)</td>
</tr>
<tr>
<td>Entrance area</td>
<td>25</td>
<td>10.80 (0-51)*</td>
<td>56 (14/25)</td>
<td>1.08 (0-10)</td>
<td>28.5 (4/14)</td>
<td>4.32 (0-48)</td>
<td>42.8 (6/14)</td>
<td>3.12 (0-40)</td>
<td>35.7 (5/14)</td>
</tr>
<tr>
<td>HS</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Patient rooms</td>
<td>100</td>
<td>3.17 (0-35)</td>
<td>37 (37/100)</td>
<td>0.92 (0-30)</td>
<td>29.7 (11/37)</td>
<td>0.51 (0-10)</td>
<td>21.6 (8/37)</td>
<td>0.62 (0-10)</td>
<td>32.4 (12/37)</td>
</tr>
<tr>
<td>Corridor</td>
<td>25</td>
<td>3.36 (0-20)</td>
<td>28 (7/25)</td>
<td>0.40 (0-10)</td>
<td>14.2 (1/7)</td>
<td>0.96 (0-10)</td>
<td>42.8 (3/7)</td>
<td>1.28 (0-10)</td>
<td>42.8 (3/7)</td>
</tr>
<tr>
<td>Hospital garden</td>
<td>50</td>
<td>3.47 (0-50)</td>
<td>20 (10/50)</td>
<td>0.40 (0-20)</td>
<td>0.26 (0-10)</td>
<td>20 (2/10)</td>
<td>2.12 (0-50)</td>
<td>70 (7/10)</td>
<td></td>
</tr>
</tbody>
</table>

*(p<0.005).
tology wards in relation with the construction and demolition works in some large buildings in order to assess the fungal load in the air, both qualitatively and quantitatively for two years. Fungus counts in that study were maximum out of the building and increased in the hematology ward from the closed (protected) toward the open areas (Pini et al., 2004). The authors proposed that, increase of the outdoor fungal concentrations almost inva-riably caused increase of the concentrations in the wards.

Level of Aspergillus was proposed by many authors to be less than 5 CFU/m$^3$ for the operating rooms and protected isolation rooms, although it is desired to be less than 0.1 to 1 CFU/m$^3$ (Perlroth et al., 2007). Considering the SCTU and HS protected areas, air contamination with Aspergillus sp. was less than 5 CFU/m$^3$ for these locations. Level of contamination was above 1 CFU/m$^3$ even in the SCTU patient rooms which were protected by HEPA filter. One of the remarkable finding of our study was that, no obvious difference in terms of the Aspergillus concentrations was found between HEPA filtered SCTU and HS where a filter of lower efficiency was used. Level of contamination in the SCTU entrance area was found above 10 CFU/m$^3$ which is accepted as the contamination level for unprotected areas. The conditions (unhealthy working of the filters, opening of the windows or insufficiency isolation, excess human entrance, doors not to be kept closed, having food in these locations, materials to be taken in with contaminated outer packages etc.) which might increase the air contamination with Aspergillus should be investigated and the necessary measures should be taken in collaboration with relevant department to decrease the contamination down to the desired levels.

In a study by Curtis et al. (2005) mean Aspergillus concentration was found as 7.2 CFU/m$^3$ in the KIT rooms with HEPA filter, higher than our results. In the study by Pini et al. (2004) which was similar to our study, mean concentrations of Aspergillus sp. were found as 1.2 CFU/m$^3$ in the rooms, 3.5 CFU/m$^3$ in the corridors and 5.6 CFU/m$^3$ in the outdoor.

Our results demonstrate that the use of ventilation systems decreases fungal contamination of the air in a certain amount. However, levels of Aspergillus contamination did not fall to zero even in the locations having HEPA filter. In practice, it is impossible to completely eliminate risk of the contamination with Aspergillus, although it can be minimized. Installation of a HEPA filtered ventilation system is not enough alone; proper maintenance and sustainability of them is also important. Air sampling is useful in order to control whether the system is still healthy.

This study was conducted in a period with lack of the activities such as construction and maintenance which produce plenty of dust, enabling us to obtain basal data for these locations of our hospital. This data would be compared with the sampling to be performed within the framework of the infection control program in the condi-

**REFERENCES**


