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A comparative Study on Mycoflora of Different Care Units in Suez Canal University Hospital, Egypt

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INTRODUCTION

To overcome nosocomial respiratory infections especially, in immunocompromised hosts, indoor air quality must be controlled. Different types of microorganisms are present in different environmental sources such as soil, dust, water, and decaying organic matter. When such materials are carried by people or air current to different healthcare units, the inhabitant microorganisms can multiply in different indoor ecological niches (Shelton et al., 2002).
Such microbes, including fungi, bacteria, and viruses, can cause airborne diseases, especially in most susceptible hosts such as elderly patients, heavy smokers, cancer patients, and solid organ transplantation patients. (Gangneux et al., 2016).

The high rates of morbidity and mortality due to the continuous increase of fungal infections in hospitals in the last few years have motivated researchers everywhere to investigate and identify bioaerosols. Studying the fungal flora in hospitals can provide full information about the epidemiology of nosocomial infectious diseases, and then controlling airborne fungi (Li & Hou, 2003; Centeno & Machado, 2004; Fernstrom & Goldblatt, 2013). Due to outbreaks of nosocomial fungal diseases, many studies have been developed in hospital environments on the fungal contamination (Dacarro et al., 2003; Vonberg & Gastmeier, 2006; Goudarzi et al., 2016; Rostami et al., 2017).

The most fungal genera isolated from the air of hospitals were Aspergillus, Penicillium, Cladosporium, and Alternaria, which were recorded as human pathogens by Rainer et al., (2000). Health hazards are caused by air fungi not only due to fungal spores but also due to their secondary metabolites. Air fungi secrete many toxic secondary metabolites that can be sheltered by their spores which can cause many problems when entering the host body (Araujo & Cabral, 2010). Therefore, it’s urgent to identify and monitor the indoor and outdoor air mycota of hospitals so, the present work aimed to determine the concentrations and diversity of fungal species in indoor and outdoor air collected from different units in a Suez Canal University Hospital.

MATERIALS AND METHODS

Sampling Sites:

The present study was conducted over one year from January 2017 to January 2018, in different four Intensive Care Units in a Suez Canal University Hospital. Indoor and outdoor air sampling, twice a month, in the first and third week of each month was performed from the Cardiothoracic Care Unit, Critical Care Unit (CCU), Intensive Care Unit (ICU), and Liver Intensive Care Unit.

Samples Collection:

Air samples from the different studied units at the hospital were performed at mid-day (hospital working time) using the settle plate method. Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) were used for fungal isolation. Chloramphenicol is an antibiotic that was incorporated into both types of media to inhibit the growth of bacteria. Samples were collected during the first and third week of each month. The plates were opened and exposed at a height of 1m to 1.5m from the floor to the air for 10 min and incubated at 28 °C for 3-5 days. After incubation, the total number of colony-forming units (CFU) was counted and converted to an organism’s colony forming unit per cubic meter (Rahkio & Korkeala, 1997; Bhatia & Vishwakarma, 2010).

The real number of fungal colonies in one cubic meter was obtained using statistical calculations. According to Gniadek & Macura (2007), CFM = a × 78.6 CFU/M$^3$

where a:- the number of fungal colonies grown from the air sample in one cubic meter of air Also, the CFU/m$^3$ air was calculated separately for each different colony observed on the plates. The average indoor temperatures during the seasons of the study period were: winter: 20±2 °C, spring: 25 ±2 °C, summer: 33 ±2 °C, and autumn: 27 ±2 °C. Fans were set off during sampling (Bhatia & Vishwakarma, 2010; Ismail et al., 2018).

Identification of Airborne Fungi:

After 4-5 days of incubation at 28 °C. All the different colonies were identified by their macro and micromorphology as the reverse colour of the mycotic colony texture and microscopic investigation, respectively (Domsch et al., 1980).

Statistical Analysis:

Data were analyzed by using a one-way analysis of variance, following Duncan's
multiple range test to determine whether there were statistically significant differences in aero-conidial concentration \((\text{CFU/m}^3)\) between the different units of Suez Canal University Hospital. \(p\)-values smaller than 0.05 were considered as statistically significant (Duncan, 1955).

**RESULTS**

The present study was conducted to determine the concentrations and types of indoor and outdoor air collected fungal species from different units in Suez Canal University Hospital. It was clear that the total colony-forming unit of fungi isolated from all the studied units was 4097.26 CFU/m\(^3\)& 4534.75 CFU/m\(^3\) which was isolated from indoor and outdoor air respectively throughout one year.

With regard to each unit separately, the CCU showed the highest percentage (31.9%) for the indoor air, followed by the liver Intensive Care Unit (29.7%), then the Cardiothoracic Care Unit (20.2%) and ICU (17.9%). This load was changed with the outdoor air, whereas the Liver Intensive Care Unit gave the highest percentage (38.20%) as shown in Table (1) & Figures (1&2).

**Table 1:** Total colony-forming units (C.F.U) and percentages of airborne fungi in the indoor and outdoor air of Suez Canal University Hospital during the study.

<table>
<thead>
<tr>
<th>Units</th>
<th>Indoor</th>
<th>Outdoor</th>
<th>Indoor</th>
<th>Outdoor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU</td>
<td>Percentage%</td>
<td>CFU</td>
<td>Percentage%</td>
</tr>
<tr>
<td>Cardiothoracic Care</td>
<td>830.33</td>
<td>20.2</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>CCU</td>
<td>1310.8</td>
<td>31.9</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>ICU</td>
<td>735.97</td>
<td>17.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver care unit</td>
<td>1220.16</td>
<td>29.7</td>
<td>38.20</td>
<td></td>
</tr>
<tr>
<td>Total counts</td>
<td>4097.26</td>
<td></td>
<td>4534.75</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1:** Total percentage of indoor airborne fungi of the different units during the entire study.
Twenty-five species (22 filamentous and 3 unicellular) were isolated and identified. The greatest total colony count was attributed to Cladosporium sp (55.8±2.38) from the Cardiothoracic Care Unit outdoor, followed by Alternaria sp (50.8±1.38) from the Liver Intensive Care Unit outdoor. Average numbers of colony-forming units (CFU) of Alternaria and Curvularia showed significant differences between various care units throughout the whole study. p ≤ 0.05 as shown in Table (2).

Table 2: Average numbers of colony-forming units (CFU) of airborne fungi in the indoor and outdoor air of Suez Canal University Hospital during the whole study

<table>
<thead>
<tr>
<th>Isolated fungi</th>
<th>Cardiothoracic Care (Indoor)</th>
<th>CCU (Indoor)</th>
<th>ICU (Indoor)</th>
<th>CCU&amp;ICU (Indoor)</th>
<th>Liver Care Unit (Indoor)</th>
<th>Mean ± St. error CFU/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherella sp</td>
<td>7.6 ± 0.14</td>
<td>7.6 ± 0.14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>4.7 ± 0.27</td>
<td>2.35 ± 0.02</td>
<td>3.14 ± 0.02</td>
<td>2.35 ± 0.02</td>
<td>0</td>
<td>2.35 ± 0.02</td>
</tr>
<tr>
<td>Pencillium sp</td>
<td>7.8 ± 0.04</td>
<td>11 ± 0.05</td>
<td>3.14 ± 0.02</td>
<td>2.35 ± 0.02</td>
<td>0</td>
<td>2.35 ± 0.02</td>
</tr>
<tr>
<td>Alternaria sp</td>
<td>0.78 ± 0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Cladosporium sp</td>
<td>30.65 ± 1.25</td>
<td>55.8±2.38</td>
<td>30.65 ± 1.25</td>
<td>55.8±2.38</td>
<td>44.4±2.08</td>
<td>47.15±1.50</td>
</tr>
<tr>
<td>Fusarium sp</td>
<td>0.78 ± 0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Scopulariopsis sp</td>
<td>0.78 ± 0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Thermomyces sp</td>
<td>0.78 ± 0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Curvularia sp</td>
<td>0.78 ± 0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Trichoderma sp</td>
<td>0.78 ± 0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Alternaria sp</td>
<td>0.78 ± 0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Rhizobolus sp</td>
<td>0.78 ± 0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Sacccharomyces cerevisiae</td>
<td>0.78 ± 0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Ulocladium sp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Neospora sp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Chaetomium sp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Rhizopus sp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Botrytis sp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Macror sp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0.00</td>
</tr>
</tbody>
</table>
During the present study, the total airborne fungal load of the Cardiothoracic Care Unit (indoor and outdoor) during one year varied from month to month. The most isolated genus from indoor and outdoor air was *Cladosporium*. The total indoor airborne fungal counts were high during December (170.29CFU/M³) and no colonies were detected in September. while outdoor airborne fungal counts were high during February (249.11CFU/m³), and also no colonies were detected in September as presented in Figure (3).

![Fig.3: Monthly variation of Cardiothoracic Care Unit total colony forming units (CFU/m³)](image)

The total indoor airborne fungal load of the Cardiovascular Care Unit peaked high indoor during November (274.8CFU/m³) and low in February (52.6 CFU/m³), also outdoor the total airborne fungal counts were high during November (209 CFU/m³) and no colonies were detected in May, July, and September as presented in Figure (4).

![Fig.4: Monthly variation of Cardiovascular Care Unit total colony forming units (CFU/m³)](image)
The total airborne fungal load of the Intensive Care Unit was assessed and the high counts of indoor fungal colonies were reported during December (170.5 CFU/m$^3$) and no colonies were detected in May, June, and September. While outdoor fungal flora showed high counts during November (209.8 CFU/m$^3$) and no colonies were detected in May and September (Fig. 5).

![Fig. 5: Monthly variation of Intensive Care Unit total colony forming units (CFU/m$^3$)](image1)

During the present study, the fungal load of liver intensive care unit showed high counts of indoor fungal colonies during February (288 CFU/m$^3$), and no colonies were detected in June and September, whereas outdoor fungal flora showed high counts during November (419 CFU/m$^3$) and no colonies were detected in June and September (Fig. 6).

![Fig. 6: Monthly variation of Liver Intensive Care Unit total colony forming units (CFU/m$^3$)](image2)
DISCUSSION

Airborne fungi in indoor environments are considered a serious problem, therefore it’s important to study their dissemination especially the pathogenic species to overcome their problem (Jaffal et al., 1997). It is regarded that the habitat where patients are treated has a critical effect on the patient's health that they may acquire an infection that can complicate their conditions (Ekhaise et al., 2010). Due to these problems, it's important to assess the quality of indoor and outdoor air in hospital environments by detecting the concentration load and the diversity of fungi present in the air.

In the current study, the concentration load and distribution of fungi in the different intensive care unit's air of Suez Canal University Hospital were assessed inside and outside of the hospital for one year. The distribution and the concentration of fungi were different between indoor and outdoor. However, the type of fungal species isolated from indoor and outdoor was the same. These investigations were confirmed by (Cho, S.Y. et al., 2018).

In this study, the most frequently isolated air fungal isolates were Cladosporium sp (55.8±.238) from the Cardiothoracic Care Unit outdoor followed by Alternaria. sp (50.8±.138) from the liver Intensive Care Unit outdoor, which concede with (Flannigan, 1994; Black 2000). Who stated that Cladosporium and Alternaria species were the most frequently distinguish fungal genera in outdoor air, also it was detected indoors. however, dissimilar with (Jaffal et al., 1997; Ekhaise & Ogboghodo 2011) who reported that Aspergillus niger and Aspergillus flavus were the most frequently isolated air fungi isolates from the indoor and outdoor environment.

The presence of Cladosporium was reported by many researchers as one of the main air pollutants in hospital environments (Lobato et al., 2009; Maldonado-Vega et al., 2014; Chaivisit et al., 2018). Cladosporium spores have allergens that can affect sensitive patient's health and can cause asthma in children (Raphoz et al., 2010).

Alternaria was also reported by Flores and Onofre (2010) as Intensive Care Unit air fungi. Different species of Alternaria was considered as an etiologic agent of cutaneous and subcutaneous diseases. Recently, A. alternata has been related to cases of cutaneous and visceral phaeohyphomycosis (Gomes et al., 2011; Raza et al., 2015).

Through this study, many different fungal species were isolated such as A. niger, A.fumigatus, Penicillium sp, and Drechslera sp this finding similar to (Ekhaise et al. 2010). Although Aspergillus may not be serious for healthy individuals, it can cause infections (aspergillosis) in immunocompromised patients (Gangneux, 2004; Bhatia & Vishwakarma, 2010). Different species of genus Aspergillus were isolated from indoor hospital environments (Kousha et al., 2011; Asif et al., 2018)

A. fumigatus was isolated from all the hospital units under the present study and undoubtedly it’s the species with the greatest effects on immunocompromised peoples (Morenogonzález et al., 2016).

Although studies on seasonal variations of microbial flora outdoor and indoor environments are rarely available, it is important for any exposure assessment (Koch et al., 2000; Pitkäranta et al. 2008. Rintala et al., 2008). From the present study, it was shown a variation in concentration load and types of isolated fungal species between outdoor and indoor. This agrees with Dubey et al., (2011)., who reported that Fungal spores are not equally distributed indoor and outdoor environments, and their distribution changes according to geographical location and meteorological conditions.

The current study explained that the total indoor and outdoor airborne fungal load of the Cardiothoracic Care Unit was high (170.29CFU/m³) during December and (249.11CFU/m³) during February, However, no colonies were detected in both outdoor and indoor in September. Also, the total airborne
fungal load of the Intensive Care Unit demonstrated high counts of indoor fungal colonies during December (170.5 CFU/ m$^3$). These data were similar to that of (Dubey et al., 2011) who reported that the highest fungal species counts were recorded in the winter season, and the lowest fungal species counts were recorded in the summer season. Also, these findings were reported by (Arora & Jain 2003).

In conclusion, the results of the current study revealed a moderate change in fungal type species between the indoor and outdoor environments. However, the concentration load of fungi was higher outdoor than indoor. Thus air exposure to fungal propagules can undoubtedly influence the health of individuals in a particular environment, especially in hospitals; this may lead to infections in different hospital units. So, hospitals should enhance the practice of good hygiene protocols to limit microbial concentration load in the air then avoid infections. Routinely monitoring the hospital mycoflora is urgently recommended. Besides care of personal hygiene, were the most effective to avoid nosocomial infections.

REFERENCES


دراسة مقارنة المحتوى الفطري بين وحدات العناية المركزية المختلفة في مستشفى جامعة قناة السويس، مصر

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يحتوي الهواء على عدد هائل من البكتيريا والفطريات وتعد تلك البكتيريا مصدر مشكلة للمرضى، حيث تعتبر الوعكة الرئيسية، وتجد هذه الوعكة بالخصوص في البيئات المغلقة. إن استنشاق الهواء الملوث يعتبر مصدر للأمراض الفطرية وخصوصاً بالنسبة للمرضى، لذلك تهدف هذه الدراسة لقياس تركيز البكتيريا والفطريات الموجودة في داخل وخارج وحدات العناية المركزية في مستشفى جامعة قناة السويس، وتجميع العينات في شهرين متواليين منisch 2017-2018 من أربع وحدات محل الدراسة مثل وحدة عناية القلب والصدر، ووحدة العناية بالقلب ووحدة العناية المركزية ووحدة العناية المركزية للكبد.

وقد أوضحت النتائج أن تركيز البكتيريا والفطريات الموجود في الهواء الداخلي والخارجي داخل هذه الوحدات كانت تصل إلى 170.29CFU/m3 & 249.11CFU/m3 خلال شهري فبراير وأغسطس، كما أن النتائج أظهرت أن الوعكة الفطرية تعتبر مشكلة كبيرة في هذه الوحدات، مع درجات أعلى في نهاية شهري فبراير وأغسطس. ونسبة العزل الهائلة تصل إلى 38.20% من البكتيريا والفطريات الموجودة في الهواء الداخلي والخارجي.

وقد أوضحت النتائج أيضاً أن تركيز البكتيريا والفطريات الموجودة في الهواء الداخلي والخارجي من وحدة عناية القلب والصدر مرتفعًا 274.8CFU/m3 & 249.11CFU/m3 خلال شهري فبراير وأغسطس. كما أن النتائج أظهرت أن الوعكة الفطرية تعتبر مشكلة كبيرة في هذه الوحدات، مع درجات أعلى في نهاية شهري فبراير وأغسطس. ونسبة العزل الهائلة تصل إلى 38.20% من البكتيريا والفطريات الموجودة في الهواء الداخلي والخارجي.

وقد أوضحت النتائج أيضاً أن تركيز البكتيريا والفطريات الموجودة في الهواء الداخلي والخارجي من وحدة عناية القلب والصدر مرتفعًا 274.8CFU/m3 & 249.11CFU/m3 خلال شهري فبراير وأغسطس. كما أن النتائج أظهرت أن الوعكة الفطرية تعتبر مشكلة كبيرة في هذه الوحدات، مع درجات أعلى في نهاية شهري فبراير وأغسطس. ونسبة العزل الهائلة تصل إلى 38.20% من البكتيريا والفطريات الموجودة في الهواء الداخلي والخارجي.