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Indoor airborne mycobiota of intensive care units in Assiut University Hospitals

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Abstract

Contamination of the air by fungi in the intensive care units (ICU) in Assiut university hospitals was investigated. Air samples were collected using settle plate method using four types of media with Rose Bengal-Streptomycin Agar. The mycobiota was isolated and identified. *Cladosporium* spp., *Aspergillus* spp., *Penicillium* spp., *Fusarium* were recorded most prevalent genera, whereas; *Alternaria* and *Scopulariopsis* were found to be with moderate frequency level. *Botryotricum*, *Circinella*, *Drechslera*, *Epicoccum*, *Ulocladium*, and others were recorded as the least fungal contaminants. The *Aspergillus* genus showed the greatest spectrum by being represented by 17 species followed by *Penicillium* (five species) .Each of *Cladosporium, Fusarium* and *Scopulariopsis* was represented by two species while the remaining 18 genera were represented by only one species for each genus**.** The gross total fungal counts of air born fungi recovered were 1009, 858, 1236 and 1366 cfu/72 plates on the four types of media used (24 exposures, 5 minutes for each exposure).

These data revealed that patients of I.C.U. in Assiut university hospitals may be in high risk due to being exposed to atmospheric air highly contaminated by those opportunistic fungal genera that may be deadly to immuno-comprmised individuals who must remain away from any airborne fungal contaminants.

Key words: Airborne fungi, Opportunistic fungi, Mycobiota

Introduction

Airborne microflora in hospital rooms was the subject of numerous studies as a potential cause of hospital infections (Gould *et al.,* 1970; Herman *et al.,* 1980; Kelsen *et al.,* 1980; Rainer *et al.,* 2001 and Li *et al.,* 2003). Some hospital infections are caused by fungi, such as species of *Aspergillus*, *Fusarium* or Mucorales (Alberti *et al.,* 2001; Faure *et al.,* 2002 and Perdelli *et al.,* 2006). Indeed, fungi present in hospital rooms may grow on the organic matter of building materials and develop microcolonies. The spores emanating from these colonies could be inhaled by immunosupressed patients and cause local infections, prior to possible dissemination (singh & Paterson, 2005).

The levels of nosocomial pathogens in the air of hospital increase due to dirtiness of air ducts by operation of the Heating, Ventilating and Air Conditioning (HVAC) system without its regular replacement (Anderson *et al.,* 1996). organic materials such as food, flower and fruit derived from outdoor environment by visitors and contamination of the interior structures by oldness of hospital (Schabrun *et al.,* 2006).

Biological contamination of indoor air is mostly caused by bacteria, moulds and yeast. They can be dangerous as pathogenic living cells but they can also secrete some health hazared materials such as mycotoxins (Flannigan *et al.,* 2001; Piecko *et al.,* 2002 and Daisey *et al.,* 2003). Fungi grow on organic matter surfaces, and their spores are dispersed in the air. While they are ubiquitous in the environment, and apparently harmless to healthy people, they can be deadly to immunocompromised individuals (Kuhn *et al.,* 2003). It is important that hospital rooms (HR) where immunocompromised patients (e.g. transplant recipient, HIV-infected or cancer patients) are placed and operating theatres (OT) remain free of airborne, opportunistic fungi. *Aspergillus* moulds are ubiquitous, and invasive aspergillosis is the most common mould infection (Vanden *et al.,* 1999).

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Materials and Methodes

Settle plate method (Hoekstra *et al*., 2004). was used to catch air-borne fungal species in the atmospheres of the air-conditioned rooms of 12 intensive care units at Assiut university hospitals during the period from June 2008 to May 2010. Four types of agar media were used for isolation of fungi (Sabouraud dextrose, Czapek's glucose, Czapek's glucose at pH 8.5, and Czapek's Cellulose agar media). Three plates of each medium were used in each room in each of 24 exposure time. Five minutes exposure time was found as an optimal time for catching a reasonable and countable number of colony forming units in intensive care units. Plates were then brought to the laboratory and incubated at 25°C ±2 for 7-10 days. Total fungal catch (TFC) for each fungus in the twenty four individual exposures (one for each month) were calculated as the number of colonies in each room.

Results

The gross total fungal counts of air born fungi recovered from 24 individual exposures in the atmosphere of different 12 air conditioned ICU in Assiut university hospitals (two exposures for each) on Czapek's glucose, Czapek's glucose at pH 85, Sabouraud dextrose and Czapek's Cellulose agar media grown at 25 ±2 °C for 7-10 days were 1009, 858, 1236 and 1366 cfu/72 plates (24 exposures, 5 minutes for each exposure), respectively Thirty seven Species belonging to 16 fungal genera were collected and identified using all the four above media

Taxa isolated are assigned to three taxonomic groups: Ascomycetes, Hyphomycetes and Zygomycetes The first group was represented by only one species (*E amestelodami*) belonging to one genus (*Eurotium*) with percentage of 217 % from the total number of fungal species The second group was the greatest number of species and represented by 40 species belonging to 17 genera accounting for 8696 % of the total number of fungal species and finally, Zygomycetes that was represented by five species belonging to five genera with percentage of 1087% from the total number of fungal species .

View on the diversity of generic level revealed that the *Aspergillus* genus showed the greatest spectrum by being represented by 17 species followed by *Penicillium* (five species) .Each of *Cladosporium, Fusarium* and *Scopulariopsis* was represented by two species while the remaining 18 genera were represented by only one species for each genus.

Eurotium amstelodami was appeared in only one case of isolation on cellulose medium.

The isolated *Aspergillus* species are belonging to 10 sections as described by Klich and Pitt (1992). Sections Nigri and Circumdati showed the greatest spectra (three species for each) Sections Flavi, Terrei and Versicolores were represented by two species for each while each of the other five sections (Candidi, Flavipes, Fumigati, Usti and Wentii) was represented by only one species . It was appeared with high frequency on all media used as airborne fungus. Frequencies of this genus were ranged from 66.67 to 100% and its densities fluctuated between 0.24-16.8%. *Aspergillus flavus*, *A. fumigates, A. awamori, A. niger* and *A. ochraceous*, *A. sydowii* were isolated from I.C.U. on all mycological media used but with different frequencies and densities. Each of *A. aculeatus, A. candidus* and *A. sulphureus* were recorded on sabouraud and cellulose agar media only.

Penicillium was represented by five species belonging to three subgenera as described by Pitt (1979) Subgenus Furcatum and Penicillium represented by two species each while subgenus Biverticillium represented by only one species. It was isolated as airborne fungus. It was represented by five species (*P. chrysogenum, P. corylophilum, P. duclauxi, P. italicum* and *P. oxalicum*). It was isolated with high or moderate frequencies in I.C.U. using the different mycological media. Its densities were ranged from 1.49 to 5.66% while their frequencies fluctuated between 33.33-62.50%.

Cladosporium was recorded with very high densities and frequencies in all the time of exposures in the atmosphere of all the 12 I.C.U. It was appeared with densities ranged from 43.04-80.3%. It was represented by *C. cladosporioides* and *C. herbarum*. The both species were isolated with high densities in

100% of isolation cases. *Fusarium* was appeared with low densities and moderate or high frequencies in all cases. Their densities were ranged from 0.93-3.44%. *Alternaria alternata* was isolated with high frequencies on Czapek's with pH 8.5 & Czapek's cellulose media and with moderate & low frequencies on Czapek's & sabouraud media, respectively, as airborne fungi in I.C.U. Stachybotrys elegans was recorded with moderate frequency on Czapek's at pH 8.5 and low on the other three media used from the air of I.C.U.

Scopulariopsis (*S. brevicaulis* and *S. brumptii*) and *Stemphylium vesicarium* were isolated as airborne fungi. Also, each of *Acremonium strictum*, *Drechslera spicifera*, *Epicoccum nigrum* and *Myrothecium roridium* were appeared as airborne fungi but with less densities and frequencies. *Ulocladium atrum* was recorded with low or rare frequencies on the four media used, while *Botryotricum piluliferum* was appeared with rare occurrence on only two types of the media.

Rhizopus stolinifer was appeared with moderate, low or rare frequencies and low desities in I.C.U. *Circinella muscae* was recorded only in one case of isolation on sabouraud medium. Finally, *Absidia corymbifera* was appeared in only one case in I.C.U. on Czapek's medium.

Discussion

A great concern has been given to the presence of airborne fungi in the hospitals as general or inside intensive care units and operation rooms in different parts of the world such as: France (Faure *et al*., 2002 and Sautour *et al*., 2009). Japan (Yonemori *et al*., 2002). Greece (Panagopoulou *et al*., 2002 and Kuleta *et al*., 2009). Thailand (Saichna *et al*., 2008). Poland (Augustowska and Dulkiewicz, 2006, Gniadek and Macura, 2007). Iran (Shadzi & Chadeganipour, 1996). Nigeria (Ekhaise *et al*., 2008). Korea (Kim *et al*., 2009). Czech Republic (Vackova *et al*., 2006). USA (Falvey and Streifel, 2007). Republic of China (Wu *et al*., 2ooo). and several other.

Indoor airborne fungi exposure frequently causes adverse human health effect with injury to and dysfunction of multiple organs and systems including: respiratory, nervous, immune, hematological systems and skin. Indoor fungi are also common causes of life threaten systematic infections in immuno-compramized patients (Curtis *et al*., 2004; Reedy *et al*., 2007). reported that fungi are important infections agents of both immuno-compromised and immuno-competent in addition to immuno-suppressed individuals.

The development of medicine, surgery and transplantology in the last thirty years has caused a dramatic increase in the number of immuno-compromised individuals who are more susceptible to fungal infections (Kuleta *et al*., 2009). Fungi cause adverse human health effects through three specific mechanisms: generation of a harmful immune response, direct infection by the organism and toxic-irritant effects from fungal metabolites (Bush *et al*., 2006).

In this study *Aspergillus* was appeared with high frequency on all media used as airborne fungus with frequenciy ranged from 66.67 to 100%, while its density fluctuated between 0.24-16.8%.

The mortality in systemic aspergillosis is high as compared with other systemic mycoses (Gniadek and Macura, 2007). Most *Aspergillus* infection occurs via inhalation. The tiny spores of this genus readily invade upper and lower airways and may produce lung aspergillosis in risk group patients (Lutz *et al*., 2003; Gangneux, 2004; Garnacho-Montereo and Amaya-villax, 2006).

Penicillium spp. was isolated as airborne fungus with high or moderate frequency on the different mycological media used and their densities ranged from 4.54 to 9.03% while their frequencies fluctuated between 58.33 and 83.33 using the four media. (Haleem khan *et al*., 2009). reported that *Penicillium* species cause infections in humans and the disease is known as penicillosis**.** It is acquired by inhalation and results in initial pulmonary infection, followed by fungemia.

Cladosporium was recorded with very high densities and frequencies in all the time of exposures in the atmosphere of all the 12 I.C.U. It was appeared with densities ranged from 43.04-80.3%. Also, it was recorded in 100% of isolation cases. It was represented by *C. cladosporioides* and *C. herbarum*. The both species were isolated with high densities in 100% of isolation cases. These results are in harmony with those recorded by (Sautour *et al*., 2009). in France. They found that in outdoor samples, *Cladosporium* was the dominant genus (55%). Also, they recorded the presence of *Cladosporium* with the highest level in spring and summer in the haematological units. (Faure *et al*., 2002). observed the presence of *Cladosporium* with 16% of total fungi in the haematologic hospital of Grenoble, France. Also Wu *et al*., (2000). isolated *Cladosporium* with 19.8% in one Asian hospital.

Fusarium was appeared with low densities and moderate or high frequencies in all cases. Their densities were ranged from 0.93-3.44% as airborne fungus in I.C.U. On the other side, their frequencies were ranged from 29.17-58.33%. It was represented by two species named *F. solani* and *F. oxysporum*.

Sautour *et al.,* (2009). isolated the airborne fungi from outdoor air and indoor two haematological units in a France hospital and recorded *Fusarium* with low frequency in all cases. *Fusarium* species were reported as causative agents of superficial and systemic infections in humans (fusariosis) (Howard, 2003). Fusarium species also produce several kinds of mycotoxins such as zearalenone, fumonisins and trichothecenes (Austen *et al.,* 2001).

The other species that had been isolated with low or rare levels, they were reported to have adverse health symptoms. (O´Hollaren *et al.,* 1991). recorded that *A. alternate* spores implicated in serious cases of respiratory arrest in Children and young adults. (Curtis *et al*., 2006). reported that the *Stachybotrys* exposed adults have noted a significantly higher incidence of health problems such as lower airway problems, wheezing, skin & eye irritation and chronic fatigue. The role of infections caused by mould *Mucor* and *Rhizopus* in severely ill patients at intensive care units is increasing. The fungi may invade patients with inhaled air as well as through the diagnostic equipment (Gniadek and Macura, 2007).

Conclusion

Generally, it is not surprising that large amounts of fungi specially belonging of *Aspergillus, Cladosporium, Penicillium, Fusarium, Alternaria, Rhizopus* and several other fungal genera were recorded in the indoor air tested of different I.C.U. in Assiut university hospitals because that fungal genera is ubiquitous. Even though it is harmless for healthy people, it may be dangerous for the patients of risk groups (mmuno-compromised and immuno-competent in addition to immuno-suppressed individuals), including those treated in surgical wards and intensive care units. Therefore, it appears that indoor air monitoring focused in the presence of these and other fungi is an important procedure in wards where risk group patients are treated. Such a procedure should be routine in Assiut university hospitals or other and particularly at intensive care units.

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الملخص العربي

الأحياء الفطرية للهواء الداخلي لوحدات العناية المركزة في مستشفيات جامعة أسيوط

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الملخص

تمت دراسة تلوث هواء وحدات العناية المركزة بمستشفيات جامعة أسيوط. تم تجميع العينات باستخدام طريقة ترسيب الهواء على أطباق باستخدام أربع أنواع من الأوساط الغذائية المحتوية على الإستربتوميسين والروزبنجال أجار. تم جمع وتعريف الأحياء الفطرية حيث تبين ان جنس الكلادوسبوريوم، الأسبرجلس، البنسليوم والفيوزاريوم الأكثر انتشارا. بينما كان جنس الألترناريا والأسكوبيولاريوبسيس الأقل بنسبه متوسطه إلى قليله أيضا جمعت مجموعه من الأخرى بنسبه قليله جدا أظهر جنس الأسبرجلس مدى واسع حيث مثل ب ١٧ نوع، يليه البنسليوم بخمسة أنواع وان كلا من الكلادوسبوريوم والفيوزاريوم والأسكوبيولاريوبسيس مثل بنوعين، بينما مثلت بقية الأجناس بنوع واحد فقط. ولقد وجد أن العدد الكلى للفطريات الناتجة كان ١٠٠٩، ٨٥٨، ١٢٣٦، ١٣٦٦ خليه على الأوساط الأربعة المختلفة المستعملة. ولقد أظهرت النتائج أن المرضى المتواجدون في وحدات العناية المركزة في مستشفيات جامعة أسيوط قد يكونوا معرضين لخطر جسيم نتيجة تعرضهم لهواء ملوث بنسبه عاليه من مجموعه من الفطريات والتي قد يكون بعضها انتهازيا، وأن مثل هذا الخطر قد يكون مميتا للأشخاص ذوى المناعة المنخفضة والمفترض تواجدهم فى جو خالي تماما من أي تلوث.