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

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## ABSTRACT

Air contains a large number of bacteria and fungi. Their assessment is critical as an indicator of contaminations for any particular environment. Inhalation of air fungal spores is considered a source of different fungal diseases, especially in immunocompromised patients. Therefore, this study aimed to compare concentrations and types of fungal species in both indoor and outdoor air of different intensive care units in a Suez Canal University Hospital. Samples were assessed twice monthly during one year from 2017-2018. Four units were investigated, Cardiothoracic Care Unit, Cardio Care Unit( CCU), Intensive Care Unit (ICU), and the Liver Intensive Care Unit. The results revealed that about 4097.26& 4534.75 colony forming units ( CFU ) were isolated from indoor and outdoor air respectively throughout the study, with a percentage of 20.2%, 27.5% from Cardiothoracic Care Unit, 31.9%, 17.1% from CCU, 17.9 %, 17.1% from ICU, and finally 29.7%, 38.20% from the Liver Intensive Care Unit from indoor and outdoor air respectively. The total airborne indoor and outdoor fungal load of the Cardiothoracic Care Unit was high 170.29CFU/$m^3$ &249.11CFU/$m^3$ during December and February respectively. The CCU isolated total fungal flora peaked high 274.8CFU/$m^3$ and 209CFU/$m^3$ indoor and outdoor respectively during November. Also, the total airborne indoor and outdoor fungal load of the ICU showed high counts of 170.5CFU/$m^3$ & 209.8CFU/$m^3$ during December and November respectively. The liver Intensive Care Unit indoor showed high counts during February 288 CFU/$m^3$. Whereas, outdoor showed high counts during November 419 CFU/$m^3$.

## 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 mycoflora 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 Cardiothorathic 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 (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.

| Units | Indoor | outdoor | Indoor | Outdoor |
|---|---|---|---|---|
| | CFU | | Percentage% | |
| Cardiothoracic Care | 830.33 | 1250.37 | 20.2 | 27.5 |
| CCU | 1310.8 | 775.99 | 31.9 | 17.1 |
| ICU | 735.97 | 775.99 | 17.9 | |
| Liver care unit | 1220.16 | 1732.4 | 29.7 | 38.20 |
| Total counts | 4097.26 | 4534.75 | | |

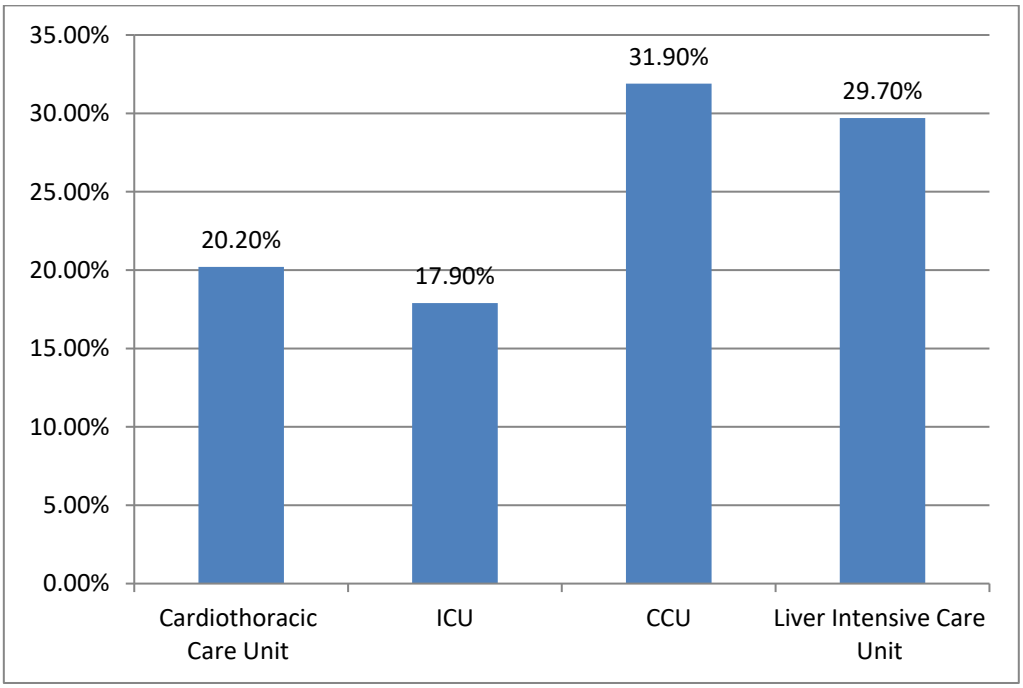


**Fig. 1:** Total percentage of indoor airborne fungi of the different units during the entire study

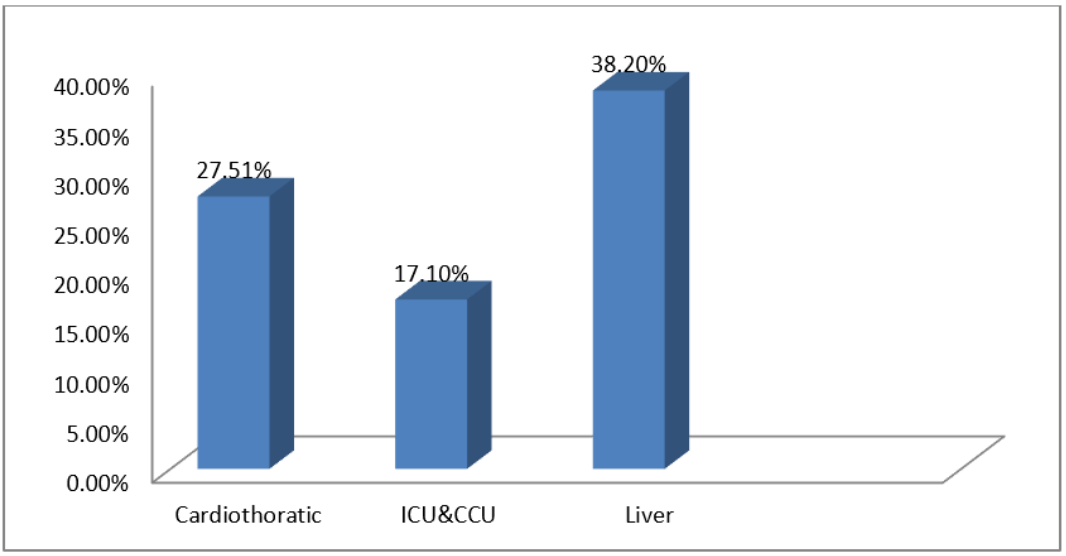


**Fig.2:** Total percentage of outdoor airborne fungi of the different units during the entire study

Twenty-five species (22 filamentous and 3 unicellular were isolated and identified. The greatest total colony count was attributed to *Cladosporium* sp (55.8±.238) from the Cardiothoracic Care Unit outdoor, followed by *Alternaria* sp (50.8±.138) 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. $p \leq 0.05$ 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

| Isolated fungi | Cardiothorathic Care | | CCU | ICU | CCU& ICU | Liver Care Unit | | P- value |
|---|---|---|---|---|---|---|---|---|
| | Indoor | Outdoor | Indoor | Indoor | Outdoor | Indoor | Outdoor | |
| | Mean ± St. error C.F.U/m$^3$ | | | | | | | |
| *Emericella* sp | .78 ±.014 | .78 ±.014 | -0 | 0 | -0 | 0- | 0- | .287 |
| *A. fluvus* | 2.35 ±.020 | 7.86 ±.060 | 2.35±.020 | 2.35±.020 | 3.14±.024 | 7.86±.053 | 3.14 ±.024 | .790 |
| *A. niger* | 8.64 ±.042 | 7.86 ±.035 | 4.71±.034 | 8.64±.073 | 6.28±.038 | 2.35±.020 | 11 ±.057 | .904 |
| *A. fumigatus* | 4.71 ±.027 | 2.35 ±.020 | .78 ±.014 | .78±.014 | .78±.014 | 2.35±.020 | 3.14±.024 | .589 |
| *Penicillium.*sp | 7.86 ±.040 | 11 ±.050 | 3.14±.024 | 6.28±.047 | 14.9± .067 | 4.71 ±.034 | 8.64 ±.061 | .208 |
| *Drechslera.*sp | 5.50 ±.030 | 4.71 ±.034 | 2.35±.020 | 3.14±.031 | 0 | .78 ±.014 | 4.71±.039 | .430 |
| *A. terreus* | 0.78 ±.014 | 0- | 0 | .78±.014 | 0 | .78 ±.014 | 2.35 ±.020 | .490 |
| *Cladosporium.*sp | 30.65±.116 | 55.8±.238 | 30.65±.124 | 31.4±.153 | 20.4±.088 | 44.8 ±.205 | 47.16±.194 | .500 |
| *Fusarium.sp* | .78 ±.014 | 3.11 ±.024 | 0 | -0 | 2.35±.028 | 0- | - | .196 |
| *Scopulariopsis.*sp | .78 ±.014 | .78 ±.014 | 0 | -0 | -0 | 0- | 3.14±.024 | .389 |
| *Stemphy-llium.* sp | .78 ±.014 | .78 ±.014 | 0- | -0 | 2.35±.020 | 4.71±.039 | - | .583 |
| *Curvularia.*sp | 0 | 3.14 ±.031 | .78 ±.014 | -0 | .78±.014 | 11 ±.082 | 7.86±.049 | .034* |
| *Trichoderma.*sp | 0 | .78 ±.014 | 0 | -0 | 0 | 0 | 0 | .646 |
| *Alternaria.*sp | 0 | 10.21±.044 | 3.14 ±.024 | 3.14±.031 | 7.86±.072 | 16.5 ±.074 | 50.8±.138 | .000* |
| *Rhodotorula.*sp | 0 | 3.14 ±.024 | 0 | 0 | 2.35±.028 | .78±.014 | 3.14±.031 | .614 |
| *Saccharomyces cerevisiae* | 0 | .78 ±.014 | 0 | 0 | 0 | 0 | 0 | .646 |
| *Ulocladium.*sp | 0 | .78±.014 | 0 | 0 | .78±.014 | 0- | 2.35±.020 | .712 |
| *Monillia.*sp | 0 | 0 | .78±.014 | -0 | 0- | 0- | 0 | .199 |
| *Eurotium.*sp | 0 | 0 | 0 | 0 | 2.35±.028 | 0 | 0 | .199 |
| *Nigrospora.*sp | 0 | 0 | 0 | .78±.014 | .78±.014 | 0 | 0 | .287 |
| *Chaetomium.*sp | 0 | 0 | 2.35±.020 | .78±.014 | 0 | 0 | 0 | .712 |
| *Rhizopus.*sp | 0 | 0 | 78±.014 | 0 | 0 | 0 | 0 | .646 |
| *Botrytis.*sp | 0 | 0 | 0 | 0 | 0 | .78±.014 | 0 | .646 |
| *Mucar.*sp | 0 | 0 | 0 | 0 | 0 | 0 | .78±.014 | .646 |
| *Candida albicans* | 0 | 0 | 0 | 0 | 0 | 0 | .78±.014 | .646 |

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/$M^3$) and no colonies were detected in September. while outdoor airborne fungal counts were high during February (249.11CFU/$m^3$), and also no colonies were detected in September as presented in Figure (3).

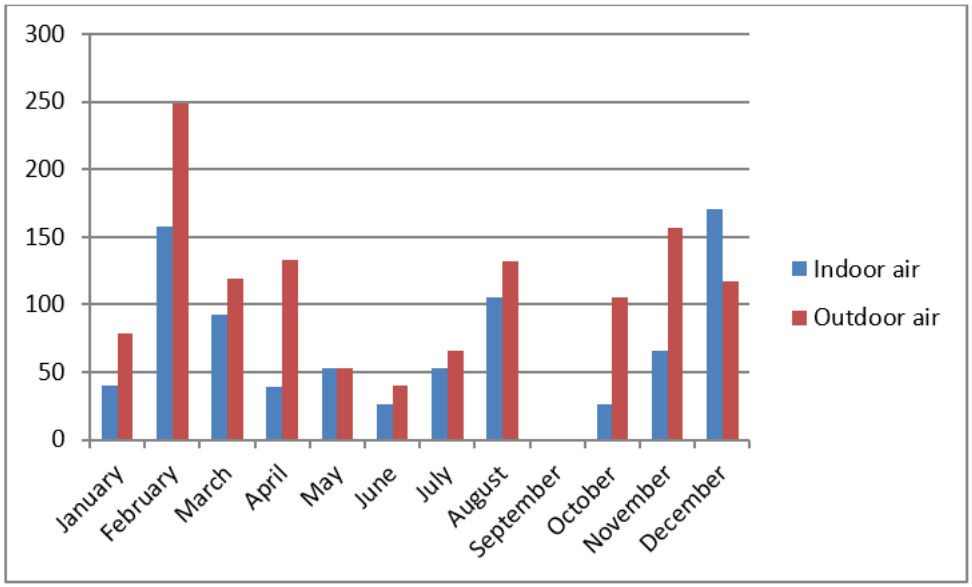


**Fig.3:** Monthly variation of Cardiothoracic Care Unit total colony forming units (CFU/$m^3$)

The total indoor airborne fungal load of the Cardiovascular Care Unit peaked high indoor during November(274.8CFU/$m^3$) and low in February (52.6 CFU/$m^3$), also outdoor the total airborne fungal counts were high during November (209 CFU/$m^3$) and no colonies were detected in May, July, and September as presented in Figure (4).

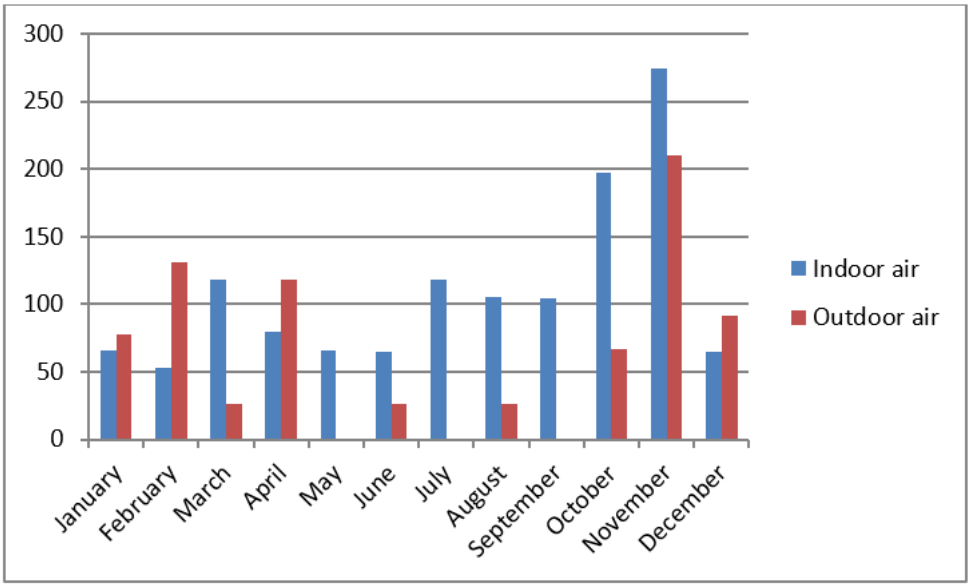


**Fig.4**: Monthly variation of Cardiovascular Care Unit total colony forming units (CFU/$m^3$)

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 (209.8CFU/$m^3$) and no colonies were detected in May and September (Fig.5).

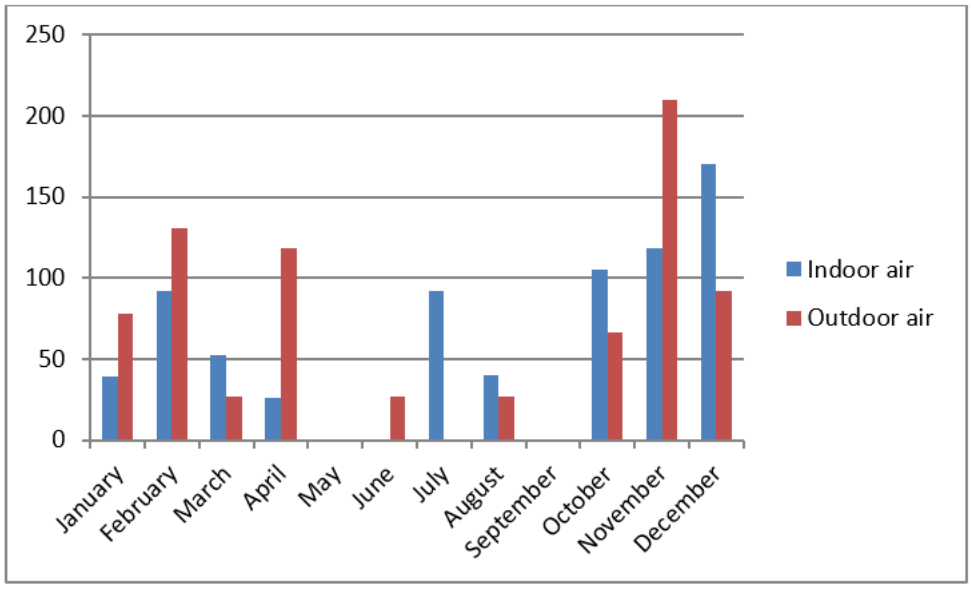


**Fig. 5:** Monthly variation of Intensive Care Unit total colony forming units (CFU/$m^3$)

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).

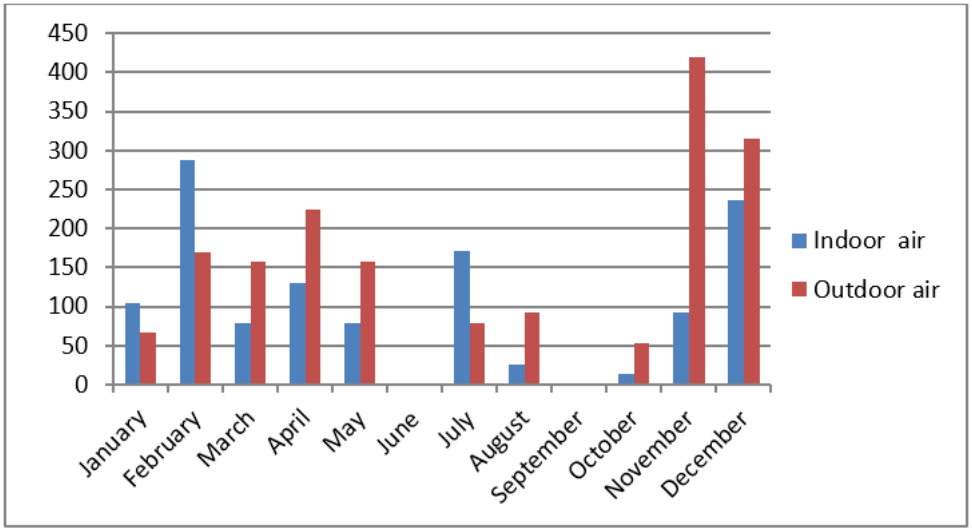


**Fig.6:** Monthly variation of Liver Intensive Care Unit total colony forming units (CFU/$m^3$)

## 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/$m^3$) during December and (249.11CFU/$m^3$) 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.

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## ARABIC SUMMARY

**دراسة مقارنة المحتوى الفطرى بين وحدات العنايه المركزه المختلفة في مستشفى جامعة قناة السويس، مصر**

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يحتوى الهواء على عدد هائل من البكتيريا و الفطريات و تقدير كم تلك الميكروبات يعتبر حيوي لأنه يعبر عن مدى التلوث الموجود فى تللك البيئه .إن إستنشاق الهواء الملوث بالميكروبات يعتبر مصدر للأمراض الفطريه و خصوصا.للمرضى.ذات المناعه الضعيفه لذلك تهدف هذه الدراسه لمقارنه تركيز و انواع الفطريات الموجوده فى داخل و خارج هواء وحدات العنايه المركزه لمستشفى جامعه قناه السويس .يتم تجميع العينات مرتين فى الشهر خلال عام من 2017-2018 من اربع وحدات محل الدراسه مثل وحدة عنايه القلب و الصدر و وحده العنايه بالقلب ووحده العنايه المركزه ووحده العنايه المركزه للكبد .و لقد اوضحت النتائج ان حوالى 4534.75 Colony Forming Units &4097.26 (CFU) تم. عزلهم من الهواء الداخلى و الهواء الخارجى للوحدات المختلفه خلال الدراسه و تتراوح نسب العزل ما بين الوحدات حيث تحتوى وحدة عنايه القلب و الصدر على27.5% ,20.2% ووحده العنايه بالقلب على 17.1% ,31.9% وو حده العنايه المركزه على 17.1% ,17.9 % و وحده العنايه المركزه للكبد على
29.7% ,38.20% من الهواء الداخلى و الهواء الخارجى للوحدات المختلفه. لقد اوضحت النتايج ايصا ان إجمالي الحمل الفطري الموجود بالهواء الداخلى و الخارجى من وحدة عنايه القلب و الصدر مرتفعًا 170.29CFU/$m^3$ &249.11CFU/$m^3$ خلال شهري ديسمبر وفبرايرو ايضا الحمل الفطري الموجود بوحده عنايه القلب 274.8CFU/m3 and 209CFU/m3 بالهواء الداخلى و الخارجى مرتفعا خلال شهر نوفبر و لقد لوحظ ارتفاعا ملحوظا فى عدد الفطريات المعزوله من الهواء الداخلى و الخارجى لوحده العنايه المركزه 209.8CFU/m3 & 170.5CFU/m3 خلال شهرى ديسمبر و نوفمبر.و اخيرا من وحده العنايه المركزه للكبد تم عزل عدد مرتفع من فطريات الهواء الداخلى 288 CFU/$m^3$. فى شهر فبراير و من الهواء الخارجى للوحده 419 CFU/m3 فى شهر نوفمبر .