Indoor Surveillance of Airborne Fungi Contaminating Intensive Care Units and Operation Rooms in Assiut University Hospitals, Egypt

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Abstract: Mycoflora of atmospheric air and dust samples collected from air conditioning systems in 12 of each I.C.U. (intensive care units) and O.R. (operation rooms) were tested using settle and dilution plate methods on four types of agar media and incubated at 25 °C. Forty-five fungal species representing 23 genera were isolated and identified. The most prevalent genera recorded were Cladosporium, Aspergillus, Penicillium and Fusarium. The total colony forming units of airborne fungi recovered in I.C.U. and O.R. ranged between 31.13-49.61 colonies/m³ on the four types of media used. The fungal total catch of the dust samples collected from the air conditioning system filters in I.C.U. and O.R. were ranged from 65.5-170 colonies/mg dust. Since, the interest to replace synthetic xenobiotics by natural compounds with low environmental persistence and biodegradable to control such airborne fungal contaminants is needed. In this respect, essential oils showed to possess a broad spectrum of antifungal activity. Fungal static ability of six oils was tested on 30 different fungal isolates. Vapors of common thyme oil exhibited the strongest inhibitory effects on the tested isolates, whereas the headspace vapors of blue gum and ginger had no inhibitory effects on the tested fungal isolates. These data revealed that the air conditioning systems may be an important source of contamination in I.C.U. and O.R. of Assiut university hospitals. Thus, patients may be in risk of being exposed to contaminated atmospheric air by opportunistic fungi and the use of essential oils as an alternative option to control hospital wards from fungal contaminants needs further studies.

Key words: Air conditions, airborne fungi, intensive care units, operation rooms and volatile oils.

1. Introduction

Recently, increased attention has been paid to fungal infections causing mycosis especially aspergillosis and therefore microorganisms in hospital rooms were the subject of many studies as the most important source of fungal infections [1-3]. Most of these studies were performed in intensive care units, surgical units, and other departments where the risk of infections is great [3-6]. Aspergillus, Fusarium and Mucor species were found to be the most common fungal genera that contaminate hospitals with low quantities ranging from 2 CFU/m³ to 26 CFU/m³ [7-10]. Indeed, fungal contamination of the hospital rooms may occur due to the growth of fungi on the organic matter of building materials. The spores emanating from these colonies could be inhaled by immunosuppressed patients and caused local infections, prior to possible dissemination [11]. Although air-conditioning systems are essential to maintain a comfortable indoor environment but also are often contaminated by microbes which their discharge into the indoor environment with strong air currents may cause fungal contamination of the air. A relation between bronchial asthma and fungal contamination by air conditioning systems was studied [12, 13]. The levels of nosocomial pathogens in the air of hospitals increased due to dirtiness of air ducts without regular replacement [14] in addition to organic
materials such as food, flowers and fruits derived from outdoor by visitors and the interior structures of the hospital [15]. The environmental fungal contamination in hospitals and the incidence of invasive aspergillosis were demonstrated by Ref. [7] and more than 500 cases of post operative aspergillosis in immunocompetent individuals were reported by Ref. [16]. *Aspergillus fumigatus* and *A. flavus* were the leading species of the genus *Aspergillus* causing invasive aspergillosis [17]. Vonberg and Gastmeier [18] reviewed all cases of invasive aspergillosis and reported that the fungus could be able to cause disease in an environment containing less than 1 CFU/m³ of air.

Essential oils showed to possess a broad spectrum of antifungal activity [19]. The advantage of plant essential oils is their bioactivity in their vapor phase which makes them used as possible antifungal fumigants [20-22].

As it is known, very limited studies of the airborne fungi in Egyptian hospitals especially I.C.U. and O.R. were performed. In this context, the aim of this study was focused on examining the spectrum and the level of fungi in the atmospheric air and the dust samples collected from the filters of their air conditioning system in Assiut university hospitals. Also, studying the antifungal activity of some essential plant oils (Blue gum, Clove, Common thyme, Ginger, Lupine and Radish) on the most prevalent fungal isolates collected was assessed.

## 2. Materials and Methods

### 2.1 Media Used

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) [23].

### 2.2 Determination of Airborne Fungi

Airborne fungi of 12 of each I.C.U. and O.R. in Assiut university hospitals were determined for two consecutive years started from June 2008 to May 2010 using Koch sedimentation method according to Polish Standard PN 89/Z-04008/08. Air mycoflora was settled directly on three plates of the four types of media per month for five minutes exposure time (optimal time for hunting a reasonable and countable number of colony forming units) in I.C.U. and 30 min in O.R. Plates were incubated at 25 ± 2 °C for 7-10 days. The fungal count was calculated as CFU/m³ according to the equation $\text{CFU/m}^3 = \text{no. of colonies} \times 10,000 / \text{surface of the Petri dish} \times \text{time of exposure} \times 0.2$ [24].

### 2.3 Determination of Mycoflora of Air-Conditioning Filter’s Dust

Dilution plate method [25-29] was used to determine the mycoflora of dust samples collected from air-conditioning filters of I.C.U. and O.R. on the four types of media used. The plates were incubated on 25 ± 2 °C for 7-10 days and TFC (total fungal colonies) were calculated.

### 2.4 Identification of Fungi

Fungal genera and species were identified on the basis of both macroscopic and microscopic characteristics identification keys of Refs. [25, 28, 30-38].

### 2.5 Essential Oils Assay

Six volatile plant oils were purchased from local markets, blue gum (*Eucalyptus globules* Labill.), clove (*Syzygium aromaticum* L. Merr & L.M. Perry), common thyme (*Thymus vulgaris* L.), Ginger (*Zingiber albus* L.), lupine (*Lupinus albus* L.) and radish (*Raphanus sativus* L.) and screened for their bioactivity on the most prevalent fungal isolates collected. Fifty mL of Czapek’s glucose agar medium [23] was poured into a sterile Petri dish and left to solidify and inoculated by the tested fungal isolate in its center. A sterile filter paper disc (≈ 2 cm) was loaded with 50 µL of the tested volatile oil and placed on the center of the upper side of the Petri dish. The Plate was sealed with polyethylene film and incubated upside down at 25 ± 2 °C for 7 days and the diameter of the fungal growth was measured in cm [39].
3. Results and Discussion

Results summarized in Tables 1 and 2 revealed that a total of 45 species belonging to 23 genera of filamentous fungi were isolated and identified from atmospheric air and dust samples collected from air conditioning systems in I.C.U. and O.R. of Assiut university hospitals. There were no appreciable differences in air mycobiota of either I.C.U. or those of O.R. as well as those of the filter’s dust samples of their air conditions. Taxa isolated were belonging to three taxonomic groups: Ascomycetes, Hyphomycetes and Zygomycetes. The first group was represented by only one species (Eurotium amstelodami), whereas the second group comprised the greatest number of fungal genera and species (39 species of 17 genera) and Zygomycetes embraced five genera and five species. The airborne total fungal forming units/m³ load in I.C.U. and O.R. were ranged from 31.13-49.61 units/m³, whereas their total fungal catch in the dust samples collected was ranged from 65.5-170 colony/mg. In one year study of fungal air contamination in outdoor and inside two hematological units in France, Sautour et al. [40] found that the mean viable fungal load was 122.1 CFU/m³ in outdoor samples and 4.1 CFU/m³ in the units. More or less similar results were reported by Ekhaise et al. [41]. They found that the fungal population in the air of five different hospital wards was ranging from 10-53 CFU/m³. Falvey and Streifel [42] reported that the mean recovery of the outdoor air was ranged from 22-122 CFU/m³, whereas the patient care areas in the hospitals in Minnesota university hospitals comprised the half number of the outdoor samples.

Although Aspergillus (17 species) and Penicillium (five species) showed the greatest spectrum of airborne fungi and dust samples of air conditions on all types of media used, but Cladosporium (two species) was recorded as the most common airborne genus (19.35-32.8 CFU/m³ and frequently appeared in 100%) followed by Aspergillus (0.97-19.93 CFU/m³ and 67%-100%) and Fusarium came third (0.12-1.71 CFU/m³ and 17%-63%). Penicillium and Rhizopus were collected with moderate to rare frequencies in I.C.U.. However, Pencillium replaced Fusarium rank in O.R. on all of the media used. Most likely, these results came in agreement with those of Sautour et al. [40] who isolated Fusarium with low frequency while Faure et al. [8] isolated Cladosporium with 16% of total fungi in the haematologic hospital in France.

In the present work, the total fungal catch units/mg dust samples Aspergillus was the most dominant genus where its total fungal count and frequency level of appearance ranged between (43.5-147 colony/mg dust and 100% respectively) followed by Fusarium (two species), whereas Penicillium occupied the third place in both I.C.U. and O.R.. All of these fungal genera were similarly recorded by Refs. [5, 8, 43-45].

Table 1  Total fungal forming units (CFU/m³) and frequency levels (F%) of fungal genera and species of airborne fungi recovered on A-Czapek’s glucose, B-Czapek’s glucose at pH 8.5, C-Sabouraud dextrose, D-Cellulose agar media in 12 of each I.C.U. & O.R. at Assiut university hospitals.

| Media used | A | B | C | D |
|------------|---|---|---|---|
| Fungal species | I.C.U.  | O.R.  | I.C.U.  | O.R.  | I.C.U.  | O.R.  | I.C.U.  | O.R.  |
| CFU | F% | CFU | F% | CFU | F% | CFU | F% | CFU | F% |
| A. corymbifera | 0.03 | 4 | | | | | | | |
| Acremonium strictum | 0.18 | 17 | 0.09 | 13 | 0.29 | 25 | 0.06 | 8 | 0.06 | 8 |
| Alternaria alternata | 0.58 | 38 | 0.21 | 25 | 0.47 | 36 | 0.15 | 17 | 0.44 | 21 |
| Aspergillus spp. | 0.97 | 67 | 5.37 | 100 | 1.83 | 67 | 3.36 | 88 | 19.93 | 100 |
| A. aculeatus | 0.09 | 13 | | | | | | | |
| A. awamori | 0.22 | 17 | 0.81 | 100 | 0.47 | 25 | 0.27 | 21 | 8.77 | 54 |
| A. candidus | 0.18 | 8 | 0.45 | 38 | 0.03 | 4 | 0.48 | 38 | 0.03 | 4 |
| A. flavipes | 0.09 | 13 | | | | | | | |
### Table 1 (continued)

| Media used                          | A.C.U. | C.R. | B.C.U. | C.R. | D.C.U. | O.R. | D.C.U. | O.R. |
|-------------------------------------|--------|------|--------|------|--------|------|--------|------|
| Parameters                          | CFU    | F%   | CFU    | F%   | CFU    | F%   | CFU    | F%   |
| Fungal species                      |        |      |        |      |        |      |        |      |
| **A. flavus**                       | 0.33    | 21   | 0.93    | 79   | 0.58    | 33   | 0.36    | 33   |
| **A. fumigates**                    | 0.03    | 4    | 0.06    | 4    | 0.06    | 8    | 0.03    | 4    |
| **A. melleus**                      | 0.18    | 8    | 0.09    | 13   | 0.27    | 21   | 0.15    | 8    |
| **A. niger**                        | 0.15    | 13   | 0.24    | 21   | 0.55    | 17   | 0.36    | 25   |
| **A. nivus**                        | 0.03    | 4    | 0.03    | 4    | 0.03    | 4    | 0.03    | 4    |
| **A. ochraceus**                    | 0.03    | 4    | 1.41    | 17   | 0.06    | 8    | 0.09    | 8    |
| **A. sulphureus**                   | 0.15    | 21   | 0.03    | 4    | 0.06    | 8    | 0.03    | 4    |
| **A. sydowii**                      | 0.15    | 17   | 1.08    | 54   | 0.11    | 13   | 0.78    | 33   |
| **A. tamarii**                      | 0.03    | 4    |         |      |         |      |         |      |
| **A. terreus**                      | 0.03    | 4    | 0.06    | 8    | 0.09    | 13   | 0.69    | 25   |
| **A. wенtii**                       | 0.06    | 4    | 0.06    | 8    | 0.03    | 4    | 0.06    | 8    |
| **A. versicolor**                   |         |      |         |      |         |      | 0.09    | 13   |
| **Botrytis cinerea** srraeae**      | 0.33    | 13   |         |      | 0.03    | 4    | 0.03    | 4    |
| **Cladosporium spp.**               | 28.74   | 100  | 27.3    | 100  | 25.06   | 100  | 24.57   | 100  |
| **C. cladosporioides**              | 18.37   | 100  | 20.01   | 100  | 16.11   | 100  | 17.67   | 100  |
| **C. herbarum**                     | 10.37   | 100  | 7.29    | 100  | 8.95    | 100  | 6.9     | 100  |
| **Cunninghamella echinulata**       |         |      |         |      | 0.15    | 13   | 0.03    | 4    |
| **Curvularia lunata**               |         |      |         |      | 0.06    | 8    |         |      |
| **Drechslera spicifera**            | 0.03    | 4    | 0.06    | 8    | 0.12    | 17   | 0.15    | 13   |
| **Epicoccum nigrum**                | 0.15    | 13   | 0.06    | 8    | 0.06    | 13   | 0.29    | 8    |
| **Eurysporum amstelodami**          | 0.09    | 13   |         |      | 0.42    | 38   | 0.03    | 4    |
| **Fusarium spp.**                   | 0.58    | 58   | 0.3     | 29   | 0.29    | 29   | 0.12    | 17   |
| **F. oxysporum**                    | 0.29    | 33   | 0.09    | 13   | 0.11    | 13   | 0.36    | 17   |
| **F. solani**                       | 0.29    | 33   | 0.21    | 13   | 0.18    | 21   | 0.12    | 17   |
| **Macrocircinelloides**             | 0.12    | 4    | 0.06    | 8    | 0.06    | 13   | 0.29    | 8    |
| **Mycorrhizum erdonium**            | 0.15    | 13   | 0.06    | 17   | 0.18    | 17   | 0.65    | 38   |
| **Penicillium spp.**                | 0.54    | 38   | 2.58    | 83   | 0.55    | 33   | 2.13    | 71   |
| **P. chrysogenum**                  | 0.15    | 17   | 0.21    | 25   | 0.15    | 17   | 0.6     | 38   |
| **P. corylophilum**                 | 0.11    | 13   | 0.72    | 50   | 0.11    | 13   | 0.69    | 54   |
| **P. dauphae**                      | 0.11    | 13   | 1.08    | 71   | 0.11    | 13   | 0.36    | 38   |
| **P. italicum**                     | 0.11    | 13   | 0.51    | 46   | 0.15    | 8    | 0.3     | 21   |
| **P. oxalicum**                     | 0.06    | 8    | 0.06    | 4    | 0.03    | 4    | 0.18    | 25   |
| **Rhizopus stolonifer**             | 0.06    | 8    | 0.03    | 4    | 0.06    | 8    | 0.15    | 21   |
| **Scopulariopsis spp.**             | 0.36    | 29   | 0.33    | 21   | 0.47    | 29   | 0.45    | 38   |
| **S. brevicaulis**                  | 0.18    | 21   | 0.21    | 17   | 0.25    | 25   | 0.27    | 33   |
| **S. brumii**                       | 0.18    | 21   | 0.12    | 4    | 0.22    | 25   | 0.18    | 17   |
| **Stachybotrys elegans**            | 0.18    | 21   | 0.33    | 38   | 0.15    | 17   | 0.15    | 13   |
| **Stemphylium vesicarium**          | 1.82    | 63   | 1.42    | 50   | 0.12    | 17   | 0.98    | 38   |
| **Talaromyces luteus**              |         |      |         |      | 0.06    | 4    | 0.06    | 8    |
| **Ulocladium atrum**                | 2.29    | 17   | 0.06    | 8    | 0.11    | 13   | 0.06    | 4    |
| **Verticilium albo-atrum**          | 0.03    | 4    |         |      | 0.09    | 13   |         |      |
| **Total CFU**                       | 36.48   |      | 36.84   |      | 31.13   |      | 31.35   |      |
| **Number of genera (23)**           | 15      | 13   | 14      | 11   | 13      | 12   | 14      | 12   |
| **Number of species (45)**          | 29      | 33   | 26      | 32   | 30      | 32   | 30      | 30   |
Table 2: Total fungal catch (TFC/mg dust) and frequency levels (F%) of fungal genera and species of air conditioning filter’s dust samples recovered on A-Czapek’s glucose, B-Czapek’s glucose at pH 8.5, C-Sabouraud dextrose and D-Cellulose agar media, in 8 of each ICU and OR at Assiut University Hospitals.

| Media used | Parameters | Fungal species    | F% | TFC | F% | TFC | F% | TFC | F% | TFC | F% | TFC | F% | TFC | F% | TFC | F% | TFC | F% |
|------------|------------|-------------------|----|-----|----|-----|----|-----|----|-----|----|-----|----|-----|----|-----|----|-----|----|
|            | A. Alternate | Alternaria alternate | 0.5 | 13  | 0.5 | 13  | 0.5 | 13  | 0.5 | 13  | 0.5 | 13  | 0.5 | 13  | 0.5 | 13  | 0.5 | 13  | 0.5 | 13  |
|            | Aspergillus spp. | 114.5 100 104.5 100 | 147 100 88.5 100 | 85.5 100 53.5 100 | 77.5 100 43.5 100 |
|            | A. awamori | 96 88 77.5 88 | 123 88 47 50 | 45 75 28 75 | 28.5 50 18 38 |
|            | A. flavus | 14 63 18.5 63 | 19 50 24.5 75 | 16 88 23.5 75 | 7 63 16 75 |
|            | A. fumigates | 0.5 | 13 0.5 | 1 | 13 | 15 | 25 | 2.5 | 25 | 0.5 | 13 | 12 | 50 | 0.5 | 13 |
|            | A. niger | 0.5 | 13 | 1 | 13 | 1 | 13 | 20 | 63 | 1 | 13 | 29.5 | 63 | 4 | 13 |
|            | A. ochraceous | 1 | 25 | 1 | 13 | 3.5 | 13 | 1 | 38 | 2 | 25 | 0.5 | 13 | 0.5 | 13 | 5 | 38 |
|            | A. tamari | 1 | 25 | 1 | 13 | 25 | 2 | 25 | 1 | 13 | 25 | 2 | 25 | 1 | 13 |
|            | A. terreus | 0.5 | 13 | 1 | 25 | 0.5 | 13 | 1 | 25 | 0.5 | 13 | 1 | 25 |
|            | A. ustus | 0.5 | 13 | 0.5 | 13 |
|            | A. versicolor | 0.5 | 13 |
|            | Fusarium spp. | 6.5 | 50 | 11 | 75 | 3.5 | 50 | 27.5 | 100 | 3.5 | 38 | 21 | 88 | 15 | 63 | 12 | 63 |
|            | F. oxysporum | 2.5 | 25 | 3 | 75 | 0.5 | 12 | 10.5 | 88 | 1 | 13 | 7.5 | 75 | 3 | 38 | 3 | 38 |
|            | F. solani | 4 | 50 | 8 | 63 | 3 | 50 | 17 | 100 | 2.5 | 38 | 13.5 | 88 | 12 | 50 | 9 | 63 |
|            | Mucor circinelloides | 1.5 | 38 | 1 | 25 | 2.5 | 25 | 0.5 | 13 | 3 | 50 | 2 | 38 | 0.5 | 25 | 1.5 | 38 |
|            | Penicillium spp. | 7 | 63 | 41 | 75 | 15.5 | 38 | 25.5 | 75 | 28 | 63 | 28.5 | 88 | 23.5 | 50 | 4 | 50 |
|            | P. chrysogenum | 1.5 | 38 | 5.5 | 75 | 4 | 25 | 10 | 50 | 3.5 | 50 | 8.5 | 50 | 3 | 38 | 3 | 38 |
|            | P. corylophilum | 1.5 | 38 | 8 | 50 | 4.5 | 25 | 3.5 | 63 | 5 | 50 | 10 | 50 | 8 | 38 | 0.5 | 25 |
|            | P. duclauxii | 1.5 | 25 | 10.5 | 50 | 3 | 38 | 2.5 | 63 | 7.5 | 25 | 3.5 | 25 | 7.5 | 38 | 0.5 | 13 |
|            | P. italicum | 2.5 | 38 | 17 | 38 | 4 | 13 | 9.5 | 50 | 12 | 38 | 6.5 | 38 | 5 | 25 | 1 | 13 |
|            | Rhizopus stolonifer | 3.5 | 13 | 1.5 | 38 | 1 | 50 | 1.5 | 38 | 3 | 50 | 1.5 | 25 | 2 | 13 | 4 | 50 |
|            | Stachybotrys elegans | 1 | 13 | 0.5 | 13 | 1 | 25 | 0.5 | 13 | 2.5 | 38 | 0.5 | 13 |
| Total TFC | 134 | 159.5 | 170 | 143.5 | 124 | 107 | 121 | 65.5 |
| Number of genera (7) | 6 | 6 | 6 | 5 | 6 | 6 | 6 | 6 |
| Number of species (19) | 18 | 15 | 14 | 13 | 14 | 14 | 14 | 14 |

Panagopoulou et al. [5] studied the environmental fungal load of air surfaces and tap water in three hospitals in different regions in Greece and found that *A. niger* was the most prevalent fungal species in the air of all hospitals followed by *A. flavus* and *A. fumigates*. In contrast, Augustowska and Dutkiewicz [46] isolated *A. fumigates* as a dominant species (77% of total fungal isolates) in air of a hospital in Poland.

Most of these fungi isolated from air or dust samples collected in this study may cause different kinds of mycosis. *Aspergillus fumigatus* is a known hazardous agent which may cause allergic alveolities, asthma, pulmonary aspergillosis and mycotoxicoses [46]. Pegues et al. [47] reported systemic infection *A. fumigatus* in patient was happened at eleven days after liver transplantation in France. Also, they found that lung aspergillosis caused by the same fungal species was detected in two patients at an intensive care unit. *Penicillium* species were reported occasionally to cause human penicillosis, pulmonary infection and fungemia [48, 49]. Moreover, Fusarium was reported as causative agents of superficial and systemic infections in humans [50].

In the second part of the study, the antifungal activity of six types of volatile plant oils (blue gum, clove, common thymus, ginger, lupine and radish) was tested on 30 selected isolates belonging to six fungal species (five/each). The results in Table 3 reflected that common thymus oil completely inhibited the growth of all fungal isolates studied but clove oil inhibited only
the growth of *C. cladosporioides* and *S. elegans*. Lupine and radish oils inhibitory action affected only *F. solani* and *C. cladosporioides* respectively. On the other hand, volatile oils of both blue gum and ginger had a partially or no inhibitory effects on all of the tested fungal isolates. Montes-Belmont and Carvajal [51] and Lee et al. [52] found similar results with those in this study. Moreover, the essential oil of thymus inhibited the growth of various fungi involved in food spoilage, mycotoxin producers, pathogenic and wood decay fungi [53, 54]. In contrast, Mourad et al. [55] found that thymus oil exhibited moderate activity against wood rot fungi. These results supported the concept that plant oils could be used as fungicidal components.

### 4. Conclusions

*Aspergillus*, *Cladosporium*, *Penicillium*, *Fusarium* and several other fungal genera were recorded in the air of different I.C.U. and O.R. in addition to the dust samples of their air conditions at Assiut university hospitals. These fungi are harmless for healthy people, but they may be dangerous for patients of risk groups, including those who are treated in O.R. and I.C.U. even if their total count will be less than 1 CFU/m$^3$ of air. Therefore, air monitoring is important in hospitals particularly in O.R. and I.C.U.. And the routine maintenance of air-conditioning systems should not be ignored. On the other hand, thymus oil had the greatest ability to inhibit the growth of all fungal isolates studied but still the use of essential oils as an alternative option to control airborne fungi that contaminate hospital wards need further studies.

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