

Seasonal fluctuations of potentially pathogenic indoor aeromycobiota in the external dermatology clinics of Assiut University Hospitals

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Abstract: In a trial to assess the aeromycobiota of the external clinics of the Department of Dermatology at Assiut University Hospitals, a preliminary survey in four sites was carried out during the four seasons of the year 2009. The sedimentation plate method and 2 forms of Sabouraud media (Sabouraud dextrose agar, SDA and Sabouraud dextrose agar supplemented with cyclohexamide, SDAC) were used. Extremely fewer total numbers of fungal propagules were obtained on SDAC than on SDA (80 versus 1059 respectively). The fungal catches showed their peak in winter on both media while the lowest number was recorded in summer on SDA and in spring on SDAC. A total of 71 species related to 26 genera were obtained on both media. The major component of airspora was *Cladosporium* (*C. cladosporioides* and *C. sphaerospermum* being the most encountered species) on both media. Other common fungi were *Aspergillus* (on SDA); *Penicillium*, *Acromonium* and *Scopulariopsis* (on SDAC). Many of the isolated fungal species are reported as human opportunistic pathogens. Therefore the implications of these mycobiota in human health are also discussed. This study also demonstrates that there is an urgent call and need for establishing guidelines on assessment and remediation of indoor mycobiota in Egyptian hospitals especially in zones at risk.

Key words: Hospital air, indoor fungi, opportunistic, cycloheximide-resistant fungi, seasonal fluctuations.

Introduction

People have continuously come in contact with airborne fungi through inhalation generally with no particular health effects. However, exposure to the potentially pathogenic fungi is a matter of health risk. Several non-pathogenic fungi are now being reported as opportunistic pathogens. Identifying both environments and fungi where people are exposed to them is of major health concern (Madisen *et al.* 2007). Previous investigations have reported that exposure to large concentrations of airborne microbes is often associated with aspergillosis (Anderson *et al.* 1996), hypersensitivity disease, such as asthma, hypersensitivity pneumonitis and rhinitis (Cuijpers *et al.* 1995, Hu *et al.* 1997) and a number of other health effects, including infections (Ren *et al.* 1999). Humans inhale approximately 200 spores of filamentous fungi per day, of which 7 reach the alveoli (Latge 1999). These spores are phagocytosed by the alveolar macrophages and destroyed (Ibrahim-Granet *et al.* 2003). As a backup defense system, polymorphonuclear leukocytes damage fungi that have escaped the effect of macrophages and germinate to hyphae. When these defense systems are defective or absent, filamentous fungi cause invasive local or disseminated infection (Roilides and Meletiadis 2003).

The hospitals and houses are continuously inhabited by human beings. Their floor dusts become heavily contaminated from different sources, particularly shoes, barefoot and/or domestic animals and indoor air flora, which settle down during the

night (Singh *et al.* 2009). Indoor fungal contamination depends on numerous factors, including construction activities, moisture, ventilation, temperature, organic matter present in building materials and the outdoor fungal load (Medrela-Kuder 2003, Oliveira *et al.* 2003). They are also influenced by seasonal and other environmental factors (Burge 1990). Studies carried out in different countries of the world provide variable results regarding the distribution and concentration of indoor fungal spores. Results have varied depending on the method used, season of the year, region and living conditions (Nolard *et al.* 2001, Ren *et al.* 2001, Chew *et al.* 2003, Trautmann *et al.* 2005).

Investigations in hospitals may also need to consider certain fungi that may be numerically rare. Such fungi pose a severe health hazard to the severely immunocompromised patients. This problem arises with special force in hospitals with bone marrow transplantation units, in hospital operating rooms, haematological and intensive care units and in patients with haematologic malignancies or with cancer, in neutropenic patients, in leukemia sufferers or major organ transplant recipients, who have the particularly severe cellular immune system deficit called neutropenia. Also, hospitals and clinics where surgery is performed on body sites particularly vulnerable to fungal infection, especially heart valves and corneas, must be especially cautious about even very small levels of opportunistic moulds (Wu *et al.* 2000, Faure *et al.* 2002, Sautour *et al.* 2009).

Consequently, the last decade has seen a significant increase in scientific data on non-

occupational exposure as well as occupational exposure to bioaerosols in many developed countries for the purpose of evaluating the relationship between exposure and health effects (Gorny and Dutkiewicz, 2002). However, for Egypt, there is only a limited amount of information currently available on the indoor fungi, including a few reports on certain public access facilities, such as water-closet environments (Ismail and Abdel-Sater 1994), subway stations (Awad 2002) and hospitals (Gharama 2007). Little is known about the seasonal distribution of fungi in hospital environments. This study was therefore undertaken: (i) to determine the concentrations of indoor fungi in the external dermatology clinics of Assiut University hospitals during the year 2009 seasons, (ii) to identify microfungi in indoor hospital air, and (iii) to evaluate the seasonal variations of the main indoor fungi identified.

Materials and methods

Sampling of airborne mycobiota

The settle plate method (a non-volumetric sampling method, referred also as sedimentation or spore trapping by deposition) (Hoekstra *et al.* 2004) was used to trap indoor airborne fungal propagules at four different sites (2 clinics, one surgery room and a hallway) of the external clinics of the Department of Dermatology at Assiut University Hospitals (see table 3) during the seasons of the year 2009. Plates (9 cm diam.) containing Sabouraud dextrose agar (SDA) and Sabouraud dextrose agar supplemented with cycloheximide (0.05%, w/v, Sigma) (SDAC) were used to catch, enumerate, and isolate the fungal propagules (4 exposures every season, 10 plates each, 5 for each medium type). The sampling time was set for 5 minutes at an approximate height of 80 cm from the floor. Sampling was performed at 11.30 am during working hours (number of people in each room at sampling was about 3-4 persons). The plates were sealed and incubated at 25 °C for 7–10 days for SDA cultures or up to one month for SDAC cultures. The growing fungi were counted, isolated and identified. The counts were expressed as colony forming units (CFUs) /5 plates in the individual exposures or the total CFUs /80 plates in each agar medium type in all exposures.

Atmospheric conditions

The average indoor temperatures during the seasons of study period were: winter: 20±2 °C, spring: 25 ±2°C, summer: 33 ±2°C, autumn: 27 ±2°C. The sites of study have no air-conditioning systems, and fans were set off during sampling.

Identification of the trapped fungi

Identification of the fungi was done on the basis of their micro- and macro-morphological characteristics using standard taxonomic keys (Raper

and Fennell 1965, Pitt 1979, Domsch *et al.* 1980, Sivanesan 1987, Moubasher 1993, de Hoog *et al.* 2000, Leslie and Summerell 2006). All the isolated fungi were deposited in the culture collection of the Assiut University Mycological Centre (AUMC), Assiut, Egypt.

Results and Discussion

A total of 71 fungal species related to 26 genera were recovered from the atmosphere of the four sites studied in the external clinics of the Department of Dermatology at Assiut University hospitals during the four seasons of the year 2009 on both isolation media used. The peak for fungal catches was recorded in winter (Tables 1 & 2).

Indoor aeromycobiota (recovered on SDA)

A total of 1059 fungal catches representing 23 genera and 60 species were recovered from 4 sites during the four seasons of study on SDA at 25 °C, with the widest spectrum of species (28) and genera (15) being recorded in Autumn.. The count peak was recorded in winter (435 catches) while the lowest number (111) was recorded in summer, i. e. the indoor fungal concentrations in winter were approximately 4 times higher than in summer. A similar finding was reported in urban and suburban homes in Taiwan (Wu *et al.* 2000). However, the fungal concentrations were significantly lower in winter than in summer in indoor air in two haematology units of a French hospital (Sautour *et al.* 2009) and at recreation facilities, elementary schools, apartments and homes in Korea (Jo and Seo 2005, Lee and Jo 2006). These differences could be related to the geographical differences in climate, and also to different sampling methods and culture medium used (Sautour *et al.* 2009).

Alternaria, *Aspergillus*, *Cladosporium*, *Eurotium*, *Penicillium* and *Rhizopus* were present throughout the four seasons, but in different loads (Table 1). The results revealed that species of *Cladosporium* (5 species, 66.10% of the total catches) were the major components of airspora with *C. cladosporioides* (36.01%) and *C. sphaerospermum* (26.44%) being the most commonly encountered species. The peaks for *Cladosporium* and its species *C. sphaerospermum* were recorded in winter; however, *C. cladosporioides* showed its maximum counts in spring. Despite the dominance of *C. herbarum* in summer, the former two species were not recorded in this season. The prevalence of *Cladosporium* species in indoor environments has also been reported by de Ana *et al.* (2006) who found that *Cladosporium* was the most dominant (followed by *Penicillium* and *Aspergillus*) in homes of fungal allergic patients and that of Wu *et al.* (2000) in Asian hospital. However *Cladosporium* came after *Aspergillus* and *Penicillium* in a french hospital (Sautour *et al.* 2009)

and after *Penicillium* in hospital operating rooms (Faure *et al.* 2002). It was common in spring and summer (Sautour *et al.* 2009) or only in summer (Medrela-Kuder 2003). Its dominance is probably linked to high spore concentrations outside, since the increase in values of fungal concentrations outside, even if modest, can directly influence indoor concentrations (Horner *et al.* 2004).

Aspergillus (9 species) and *Penicillium* (15) came after *Cladosporium* in their counts. They accounted for 17.28% and 6.80% of the total catches respectively. Their maxima were recorded in summer and winter and their minima in spring and summer, respectively. The most encountered aspergilli were *A. niger* (10.20%) followed by *A. flavus* (3.97%) and *A. sydowii* (1.51%) exhibiting their peaks in winter, summer and autumn, respectively. Of the 15 *Penicillium* species identified, only *P. chrysogenum* (2.46%) and *P. aurantiogriseum* (1.32%) were dominant showing their peaks in autumn and winter, respectively. Other aspergilli and penicillia were recorded sporadically in one or two seasons and constituted a minor component of the airspora in the sites of study. In contrast to our finding, *Aspergillus* (64.2% of the total CFU) was the most common fungus in the air of a tertiary care hospital in Greece with *A. niger* (26%), *A. flavus* (17.9) and *A. fumigatus* (12.4%) being the most encountered, while *A. terreus* and *Emericella nidulans* were also recorded but less frequently (Panagopoulou *et al.* 2007). *A. flavus* was the most prevalent *Aspergillus* species to be recovered from the air of hospital wards and homes in Iran (Hedayati *et al.* 2005). Outbreaks of *Aspergillus* involving the skin, oral mucosa or subcutaneous tissues are more often associated with *A. flavus* than other species (Heinemann *et al.* 2004). During their study of aerobiology of fungi in Palencia, Spain, Herrero *et al.* (1996) found that spores of *Aspergillus* were predominant in winter while those of *Penicillium* in the first-half of the year. In Cracow, Poland, both *Aspergillus* and *Penicillium* predominated in autumn and winter (Medrela-Kuder 2003). In contrast to our finding, species of *Penicillium* and *Aspergillus* were the most frequently found fungi, followed by species of *Cladosporium*, *Alternaria* and/or *Bjerkandera* in haematological units of a French hospital (Sautour *et al.* 2009), Dijon hospital (Sautour *et al.* 2007), in one of the Taiwani hospitals (Wu *et al.* 2000) and in newborn babies homes in Paris, France (Dassonville *et al.* 2008). It was reported that the spores of members of *Aspergillus* were the sources of infection in numerous outbreaks of nosocomial aspergillosis (Cheng and Streifel 2001, Vonberg and Gastmeier 2006). There is no consensus on a threshold spore concentration at which a significant risk of fungal infection occurs (Vonberg and Gastmeier 2006), but

it had been shown that peaks in air contamination by *Aspergillus* spores > 2 CFU/m³ were correlated with an increase risk of invasive aspergillosis (Alberti *et al.* 2001).

Other less frequent fungi were *Alternaria alternata* (1.23%), *Rhizopus* (1.13%), *Epicoccum nigrum* and *Stemphylium botryosum* (0.94% each). They were recovered either during all seasons or at least in 2 seasons of study, giving their peaks in winter except, *Rhizopus* in spring. Species of dematiaceous hyphomycetes (e. g. *Drechslera* and *Alternaria*), dermatophytes, Zygomycetes and other filamentous fungi were isolated from the air of a tertiary care hospital in Greece (Panagopoulou *et al.* 2007). *Alternaria* showed also the highest concentration during the autumn in a French hospital and this was attributed to the presence of numerous spores in outdoor at that period of the year (Sautour *et al.* 2009).

The four most prevalent fungal genera detected in our study (*Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*) have also been reported to be common in the air of residential environments in different countries (Dharmage *et al.* 1999, Ren *et al.* 1999, 2001, Chew *et al.* 2003, Horner *et al.* 2004, de Ana *et al.*, 2006, Lee and Jo 2006) and in the indoor air of schools and offices in Denmark (Gravesen *et al.* 1986), and in a hospital (Hong *et al.* 2003), bars, internet cafés, elementary schools and homes (Jo and Seo 2005), apartments (Lee and Jo 2006) in Korea. These genera are considered to be most relevant for allergies (de Ana *et al.* 2006).

In the study of de Ana *et al.* (2006) on seasonal distribution and prevalence of fungi in homes of fungal allergic patients in Spain, the highest presence of *Aspergillus*, *Cladosporium* and *Penicillium* was registered in autumn, while *Alternaria* was more frequent in summer, with the largest number of isolations were of *Cladosporium* and *Penicillium* during the four seasons. They also found that the most prevalent species are *A. alternata*, *C. herbarum*, *C. cladosporioides*, *A. niger* and *P. chrysogenum*. In Addition, *A. flavus*, *A. versicolor*, *P. frequentans* and *P. funiculosum* were also recorded.

The remaining 26 fungal species in addition to some yeasts and non-sporulating fungi were recorded sporadically and infrequently, accounting collectively for 5.63% of the total catch (Table 1).

Table 1: Indoor mycobiota recovered on Sabouraud agar (SDA) from hospital air in different seasons of the year 2009.

Taxa	Winter		Spring		Summer		Autumn		Total	
	Count	%	Count	%	count	%	count	%	count	%
<i>Acremonium kiliense</i> Gütz							1	0.4	1	0.09
<i>Alternaria alternate</i> (Fries) Keissler	7	1.61	2	0.77	3	2.7	1	0.4	13	1.23
<i>Aspergillus</i> (Total)	54	12.41	27	10.3	60	54.1	42	16.67	183	17.28
<i>A. flavus</i> Link	7	1.61	13	4.98	16	14.4	6	2.83	42	3.97
<i>A. flocculosus</i> Frisvad & Samson							1	0.4	1	0.09
<i>A. fumigatus</i> Fresenius	1	0.23			2	1.8			3	0.28
<i>A. niger</i> van Tieghem	41	9.43	10	3.83	34	30.6	23	9.13	108	10.20
<i>A. ochraceus</i> Wilhelm					3	2.7	3	1.2	6	0.57
<i>A. sydowii</i> (Bainier & Sartory) Thom & Church	2	0.46	3	1.15	4	3.6	7	2.78	16	1.51
<i>A. tamarii</i> Kita	1	0.23							1	0.09
<i>A. terreus</i> Thom	2	0.46					1	0.4	3	0.28
<i>A. ustus</i> (Bainier) Thom & Church			1	0.38			1	0.4	2	0.19
<i>A. versicolor</i> (Vuillemin) Tiraboschi					1	0.9			1	0.09
<i>Candida</i> sp.							5	1.98	5	0.47
<i>Cladosporium</i> (Total)	316	72.64	201	77	28	25.2	154	61.11	699	66.01
<i>C. cladosporioides</i> (Fresenius) de Vries	54	12.41	192	73.6			136	53.97	382	36.10
<i>C. herbarum</i> (Persoon) Link					22	19.8			22	2.08
<i>C. oxysporum</i> Berk. & Curt.			8	3.07					8	0.76
<i>C. sphaerospermum</i> Penzig	262	60.23					18	7.14	280	26.44
<i>Cladosporium</i> sp.			1	0.38	6	5.41			7	0.66
<i>Cochliobolus</i> (Total)			1	0.38	1	0.9	1	0.4	3	0.28
<i>C. lunatus</i> R. Nelson & Haasis					1	0.9			1	0.09
<i>C. spicifer</i> Nelson			1	0.38			1	0.4	2	0.19
<i>Cylindrocladium parvum</i> Anderson							1	0.4	1	0.09
<i>Emericella</i> (Total)					1	0.9	1	0.4	2	0.19
<i>E. heterothallica</i> (Kwon <i>et al.</i>) Malloch & Cain							1	0.4	1	0.09
<i>E. nidulans</i> (Eidam) Vuillemin					1	0.9			1	0.09
<i>Epicoccum nigrum</i> Link	7	1.61	1	0.38	2	1.8			10	0.94
<i>Eurotium</i> sp.	5	1.15	1	0.38	1	0.9	1	0.4	8	0.76
<i>Exserohilum heteropogonicola</i> Sivan.			1	0.38					1	0.09
<i>Fusarium</i> (Total)			3	1.15	1	0.9			4	0.38
<i>F. proliferatum</i> (Matsushima) Nirenberg			2	0.77					2	0.19
<i>Fusarium</i> sp.			1	0.38	1	0.9			2	0.19
<i>Geotrichum candidum</i> Link							1	0.4	1	0.09
<i>Neurospora crassa</i> Shear & Dodge					1	0.9			1	0.09
<i>Paecilomyces</i> (Total)	3	0.69							3	0.28
<i>P. clavissporus</i> Hammill	1	0.23							1	0.09

Taxa	Winter		Spring		Summer		Autumn		Total	
	Count	%	Count	%	count	%	count	%	count	%
<i>P. variotii</i> Bainier	2	0.46							2	0.19
<i>Penicillium</i> (Total)	29	6.7	8	3.07	7	6.31	28	11.11	72	6.80
<i>P. aurantiogriseum</i> Dierckx	13	3			1	0.9			14	1.32
<i>P. brevicompactum</i> Dierckx							4	1.59	4	0.38
<i>P. chermisinum</i> Biourge	1	0.23							1	0.09
<i>P. chrysogenum</i> Thom	9	2.07			1	0.9	16	6.35	26	2.46
<i>P. citrinum</i> Thom					1	0.9			1	0.09
<i>P. duclauxii</i> Delacroix	1	0.23					2	0.8	3	0.28
<i>P. expansum</i> Link	1	0.23							1	0.09
<i>P. funiculosum</i> Thom			3	1.15					3	0.28
<i>P. glabrum</i> (Wehmer) Westling							2	0.8	2	0.19
<i>P. italicum</i> Wehmer							1	0.4	1	0.09
<i>P. puberulum</i> Bainier	2	0.46							2	0.19
<i>P. restrictum</i> Gilman & Abbott					1	0.9			1	0.09
<i>P. spinulosum</i> Thom							2	0.8	2	0.19
<i>P. variabile</i> Sopp					1	0.9	1	0.4	2	0.19
<i>P. verrucosum</i> Dierckx			3	1.15					3	0.28
<i>Penicillium</i> spp.	2	0.46	2	0.77	2	1.8			6	0.57
<i>Phoma herbarum</i> Westendorp							1	0.4	1	0.09
<i>Rhizopus</i> (Total)	2	0.46	5	1.92	3	2.7	2	0.8	12	1.13
<i>R. oryzae</i> Went & Prinsen-Geerligs			1	0.38					1	0.09
<i>R. stolonifer</i> (Ehrenberg) Vuillemin	2	0.46	4	1.53	3	2.7	2	0.8	11	1.04
<i>Rhodotrula</i> sp.							3	1.19	3	0.28
<i>Scopulariopsis</i> (Total)			1	0.38			5	1.98	6	0.57
<i>S. brevicaulis</i> (Saccardo) Bainier							1	0.4	1	0.09
<i>S. brumptii</i> Salvanet- Duval							4	1.59	4	0.38
<i>Scopulariopsis</i> sp.			1	0.38					1	0.09
<i>Setosphaeria rostrata</i> Leonard	1	0.23							1	0.09
<i>Stemphylium botryosum</i> Wallr.	7	1.61					3	1.19	10	0.94
<i>Trichoderma</i> (Total)	1	0.23					1	0.4	2	0.19
<i>T. reesei</i> Simmons							1	0.4	1	0.09
<i>Trichoderma</i> sp.	1	0.23							1	0.09
<i>Ulocladium</i> (Total)			2	0.77	1	0.9			3	0.28
<i>U. atrum</i> Preuss					1	0.9			1	0.09
<i>U. botrytis</i> Preuss			2	0.77					2	0.19
Non-sporulating fungi			3	1.15	1	0.9	1	0.4	5	0.47
Yeasts	3	0.69	5	1.92	1	0.9			9	0.85
Total	435	100	261	100	111	100	252	100	1059	100
Number of genera	11		13		12		15		23	
Number of species	25		21		23		28		60	

Counts are calculated as colony forming units (CFU) per 4 exposures (5 agar plates and 5 min. exposure each)

Cyclohexamide-resistant indoor aeromycobiota (recovered on SDAC):

Extremely fewer numbers of propagules (80) were recovered from the indoor hospital air on Sabouraud dextrose agar amended with cyclohexamide (0.05%, w/v) compared to those recovered on cyclohexamide-free medium (1059). The highest number of propagules was caught in winter whereas the lowest was in spring (Table 2). These propagules were classified into 27 species belonging to 12 genera of which *Cladosporium* (30% of the total catches) followed by *Penicillium* (15%), *Acremonium* and *Scopulariopsis* (13.75% each) were the most common. Their peaks were recorded in winter. The dominant species of these fungi are *Cladosporium sphaerospermum*, *C. cladosporioides*, *Penicillium chrysogenum*, *P. aurantiogriseum*, *Acremonium strictum*, *Scopulariopsis brevicaulis* and *S. brumptii*. In addition, *Alternaria* (*A. alternata*) and *Aspergillus* (4 species) came behind these genera according to their counts.

Interestingly, nine fungal species were recovered only on cycloheximide-containing medium infrequently and sporadically. These are *Acremonium fusidioides*, *A. strictum*, *Aspergillus fumigatus* var. *albus*, *Humicola fuscoatra*, *Pseudoallescheria boydii*, *Scopulariopsis candida* and the keratinophilic species *Chrysosporium keratinophilum*, *C. pannicola* and *C. tropicum*.

It has been reported that *C. tropicum* is a probable cause of dermatomycoses, *C. keratinophilum* is isolated repeatedly from onychomycoses and superficial infections, *C. pannicola* is found to be involved in superficial skin infection in human and dogs, *Pseudoallescheria boydii* as an agent of white-grained mycetoma, cutaneous infection and central nervous system infection, *Acremonium strictum* causes invasive infection in neutropenic patients (de Hoog *et al.* 2000, Kushwaha 2000).

Other sporadic fungi reported from indoor hospital air are listed in table (2).

Correlation between aeromycobiota and sites and seasons of sampling

The trend of the total colony forming units (CFU) inside the two clinics was almost similar, where the fungal spore load was far higher in winter (172, 134) than in spring (67, 61), autumn (64, 44) and summer (30, 29) as shown in Table (3). The same seasonal trend was observed in the surgery room, however, lower CFU were obtained in winter (74 CFU) compared to those from the two clinics. A different trend was observed in the corridor where the highest CFU was recorded in autumn and the lowest in summer. The data also show that the most predominant species in the four sites of study was *Cladosporium sphaerospermum* in winter season, *C.*

cladosporioides in both spring and autumn, and *Aspergillus niger* in summer. However, the second most common species was almost different not only from one site to another but also from one season to another, e. g. in winter it was *C. cladosporioides* in clinic 1, *Penicillium aurantiogriseum* in clinic 2, and *A. niger* in both surgery room and the corridor (hallway). The second most common species was *C. herbarum* in clinic 1 in summer, *A. niger* in clinic 2 in summer, and *C. oxysporum* in both clinics in spring. Meanwhile, *A. flavus* was the second in both the surgery room and corridor in spring and summer, *A. niger* in clinic 2 and surgery room while *C. sphaerospermum* in clinic 1 and *P. chrysogenum* in the corridor (Table 3). Despite the performance of the current work in dermatological clinics, it is noted that none of the dermatomycobiota species was recorded, this may be explained that these fungi have no ability to be suspended in the air. It is noted also that the sites of study did not show high damp levels, as shown by the absence or the low frequency of water-indicator fungi such as *Chaetomium* and *Stachybotrys* (absent), and *Fusarium* and *Ulocladium* (low) as indicated by Dillon *et al.* (1999) and Hicks *et al.* (2005).

It is note-worthy that some fungal species trapped in the current study such as *A. fumigatus*, *A. niger*, *A. flavus* and *Fusarium* spp. have been reported to be infectious under immunocompromised conditions. Others are involved in human diseases in the respiratory system due to inhalation of spores of *Cladosporium* spp. *Alternaria* spp. (Srivastava and Wadhvani 1992) or *Aspergillus* (*A. fumigatus*, *A. flavus*, *A. niger*, *A. nidulellus* and *A. terreus*) and *Penicillium* (Pitt 1994). Some species of *Alternaria* have been also reported to cause onychomycosis and phaeoerythromycosis (de Hoog *et al.* 2000).

Conclusion, suggestions and recommendations

Results of the present work especially those obtained on SDA highlight the elevated number of isolated colonies and their distribution in 23 genera and 60 species of fungi, with the great majority of these colonies (91.31%) belonging to the four genera, that are considered the causative agents of a large number of respiratory allergies, in the following order: *Cladosporium*, *Aspergillus*, *Penicillium* and *Alternaria*.

The four genera were recorded all year round, however, the peaks for *Cladosporium*, *Penicillium* and *Alternaria* were in winter while, that for *Aspergillus* was in summer.

Fungi resistant to cyclohexamide include some species not encountered on SDA (cyclohexamide-free) such as the keratinophilic species *C. keratinophilum*, *C. pannicola* and *C. tropicum* and the opportunistic pathogens, *Acremonium fusidioides*, *A. strictum*, *Pseudoallescheria boydii* and

Table 2: Cyclohexamide-resistant indoor mycobiota recovered from hospital air during different seasons on Sabouraud agar amended with cyclohexamide (SDAC).

Taxa	Winter		Spring		Summer		Autumn		Total	
	count	%	count	%	count	%	count	%	count	%
<i>Acremonium</i> (Total)	8	16.33	1	50			2	18.18	11	13.75
<i>A. fusidioides</i>	2	4.08							2	2.5
<i>A. kiliense</i>	1	2.04							1	1.25
<i>A. strictum</i>	1	2.04	1	50			2	18.18	4	5.0
<i>Acremonium</i> sp.	4	8.16							4	5.0
<i>Alternaria alternata</i>	1	2.04			1	5.56	3	27.27	5	6.25
<i>Aspergillus</i> (Total)	4	8.16			1	5.56			5	6.25
<i>A. flavus</i>	1	2.04							1	1.25
<i>A. fumigatus</i> var. <i>albus</i>					1	5.56			1	1.25
<i>A. niger</i>	1	2.04							1	1.25
<i>A. sydowii</i>	2	4.08							2	2.5
<i>Candida</i> sp.					1	5.56			1	1.25
<i>Chrysosporium</i> (Total)			1	50	2	11.11	1	9.09	4	5.0
<i>C. keratinophilum</i>					1	5.56			1	1.25
<i>C. pannicola</i>					1	5.56			1	1.25
<i>C. tropicum</i>			1	50			1	9.09	2	2.5
<i>Cladosporium</i> (Total)	14	28.57			8	44.44	2	18.18	24	30.0
<i>C. cladosporioides</i>	8	16.33			2	11.11			10	12.5
<i>C. sphaerospermum</i>	6	12.24			5	27.8	1	9.09	12	15.0
<i>C. colocasiae</i>							1	9.09	1	1.25
<i>Cladosporium</i> sp.					1	5.56			1	1.25
<i>Emericella nidulans</i>					1	5.56			1	1.25
<i>Humicola fuscoatra</i>	1	2.04							1	1.25
<i>Penicillium</i> (Total)	12	24.48							12	15
<i>P. aurantiogriseum</i>	4	8.16							4	5.0
<i>P. chrysogenum</i>	7	14.29							7	8.75
<i>Penicillium</i> sp.	1	2.04							1	1.25
<i>Pseudallescheria boydii</i>					1	5.56			1	1.25
<i>Scopulariopsis</i> (Total)	7	14.29			1	5.56	3	27.27	11	13.75
<i>S. brevicaulis</i>	3	6.12					1	9.09	4	5.0
<i>S. brumptii</i>	2	4.08			1	5.56	1	9.09	4	5.0
<i>S. candida</i>	2	4.08					1	9.09	3	3.75
<i>Trichoderma</i> sp.					1	5.56			1	1.25
Yeasts	2	4.08			1	5.56			3	3.75
Total	49	100	2	100	18	100	11	100	80	100
Number of genera	7		2		9		5		12	
Number of species	17		2		12		8		27	

Table 3: The most common species (recovered on SDA) during the different seasons in the different sites .

Season	Winter		Spring		Summer		Autumn	
Site of collection (CFU)	Common species (CFU)	Total CFU	Common species (CFU)	Total CFU	Common species (CFU)	Total CFU	Common species (CFU)	Total CFU
Clinic 1 (333)	<i>C. sphaerospermum</i> (141) <i>C. cladosporioides</i> (6)	172	<i>C. cladosporioides</i> (45) <i>C. oxysporium</i> (5)	67	<i>A. niger</i> (8) <i>C. herbarum</i> (7)	30	<i>C. cladosporioides</i> (35) <i>C. sphaerospermum</i> (5)	64
Clinic 2 (268)	<i>C. sphaerospermum</i> (102) <i>P. aurantiogriseum</i> (7)	134	<i>C. cladosporioides</i> (45) <i>C. oxysporum</i> (4)	61	<i>C. herbarum</i> (12) <i>A. niger</i> (4)	29	<i>C. cladosporioides</i> (24) <i>A. niger</i> (6)	44
Surgery room (208)	<i>C. sphaerospermum</i> (34) <i>A. niger</i> (22)	74	<i>C. cladosporioides</i> (50) <i>A. flavus</i> (4) <i>A. niger</i> (4)	63	<i>A. niger</i> (14) <i>A. flavus</i> (3)	28	<i>C. cladosporioides</i> (18) <i>A. niger</i> (6)	43
Corridor (250)	<i>C. sphaerospermum</i> (24) <i>A. niger</i> (10)	55	<i>C. cladosporioides</i> (52) <i>A. flavus</i> (4)	70	<i>A. niger</i> (8) <i>A. flavus</i> (5)	24	<i>C. cladosporioides</i> (63) <i>P. chrysogenum</i> (10)	101
Total (1059)		435		261		111		252

CFU = colony forming units per 5 agar plates, 5 min. exposure each.

Scopulariopsis candida. However none of the dermatomycobiota were recorded on both media.

The present survey indicates that indoor air of hospital harbors some potentially human pathogenic, keratinophilic and other opportunistic fungi.

It is neither possible nor warranted to eliminate all indoor fungal spores and hyphal fragments; however, mold growth indoors can and should be prevented and removed using suitable methods of cleaning and disinfection.

Despite the valuable informations obtained in the current study on the fungi spectrum as well as their concentrations and dominance, for the first time in such area of Assiut University Hospitals, it is recommended to use the volumetric methods, which was not available during this study, to be able to compare our data (on fungal concentrations) with the specified guidelines, which are between 100 and 1000 CFU m⁻³, as suggested by the American Conference of Government Industrial Hygienists (ACGIH, 1989) and/or of the Korean indoor bioaerosol guidelines (800 CFU m⁻³) (Jo and Seo 2005). We introduce a suggestion to the Ministry of Environment to apply these specified guidelines (between 100-1000 CFU m⁻¹) or less.

Establishing guidelines on assessment and remediation of hospital indoor aeromycobiota particularly in zones at risk such as intensive care units, transplant and haematological units and operating theatres is urgently needed. In this respect, a suggestion to start evaluating hospital indoor and outdoor airspora using both volumetric and non-volumetric methods, different culture media and incubation temperatures is raised. Moreover, it is highly recommended to plan for periodic surveys of the indoor aeromycobiota in hospitals, houses, work places, schools, study centers and offices for continuous monitoring of the prevalence of potentially pathogenic fungi.

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