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Indoor air studies of fungi contamination at the Department of Pulmonology and Internal Medicine in Kavala Hospital in Greece

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ABSTRACT

Purpose: The aim of the study was to assess the presence of airborne fungi and the fungal flora of the walls in the Departments of Pulmonary and Internal Medicine in Kavala Hospital (Greece).

Material and Methods: The study was carried out at the Department of Pulmonology and Internal medicine in Kavala Hospital. Materials for the tests were: the air samples (in front of the building and the selected rooms) and swabs from the walls. The air pollution was determined using SAS SUPER 100 (Pbi International). The microbial flora from walls was assessed using the Count-Tact applicator and the plate Count-Tact (BioMerieux). Humidity and temperature were evaluated by a thermo-hygrometer.

Results: The following fungal pathogens isolated from air were *Aspergillus, Candida albicans, Candida spp*., and *Penicillium species* in the Department of Pulmonary. Similar pathogens in the air of Department and Internal Medicine were found. Mean number of fungi colonies isolated from air in the Department of Pulmonology was significantly ($p<0.001$) higher compared to the Department of Internal Medicine. No significant correlations between CFU of fungi in air and temperature in both Departments were found.

Conclusions: The main fungal pathogens isolated from the air samples were *Aspergillus* and *Candida albicans* in the Departments of Pulmonary and Internal Medicine in Kavala Hospital. Fungal occurrence in the air of rooms and walls varied between the both departments of the same hospital.

Key words: indoor air fungi, hospital, SAS Super 100

INTRODUCTION

Fungal infection have emerged as important causes of morbidity and excessive mortality in immunocompromised patients [1,2]. Many of these infections are endogenous in nature, but others can be acquired by exogenous routes, through the hands of healthcare workers, contaminated infusion products and biomaterials [2,3]. Nosocomial fungal infection remains an important problem in the hospital departments. Hospital wards had been shown to act as reservoirs of pathogenic microorganisms. *Aspergillus fumigatus* is ubiquitous in the environment. It is also a major human pathogen, responsible for allergic diseases, aspergilloma and invasive aspergillosis in immunocompromised patients. A high mortality (usually over 50%) of aspergillosis is accompanied by the high costs of treatment [4,5]. Furthermore, some fungi such as *Aspergillus versicolor* and *Stachybotrys chartarum* are able to produce mycotoxins [5,6]. The risks factors that predispose people to invasive aspergillosis include neutropenia, artificial pulmonary ventilation, long-term administration of immunosuppressive drugs (e.g. corticosteroids), and broadspectrum antibiotics [6].

To our knowledge no study on environmental contamination by airborne fungi in the Departments of

Pulmonary and Internal Medicine in Kavala Hospital (Greece) was performed.

The aim of the study was to assess the presence of airborne fungi and the fungal flora of the walls in the Departments of Pulmonary and Internal Medicine in Kavala Hospital.

MATERIAL AND METHODS

Air sampling were carried out in the Departments of Pulmonary and Internal Medicine in Kavala Hospital (Greece) in May 2008. Material into mycological studies was air sampled at the entrance of hospital building, the entrance into operating room, hall and the selected rooms of operating department and nurses' stations. Detection of airborne fungi outside hospital is very important due that the immunocompromised patients get colonized outside and they bring in their own isolates. The monitoring of airborne fungi pollution was done using a SAS SUPER 100 (pbi international) with international measure standards (EN 50081-1, EN 500 50082-1). Sample has a flow rate of 100 liters air/min. At each site, a 100 liters sample was taken with the sampler placed at a height of 150 cm above floor level in the middle of the room, with all windows and doors closed. Plates from SAS SUPER 100 were incubated. After incubation number of fungal colonies and number of fungi in air volume was counted. In according to producer, at the first part of investigation number of fungal colonies at plates (real number of colonies- RNC) was corrected on statistical probability multiple passage of particle through the same hole (number of colonies corrected). In according to formula, it was estimated CFU (number of colonies at 1000 L of air): X = (P x 1000) : V, where : V- volume of air sample , r – number of counted colonies at contact plate, P - corrected number of colonies (in according to producer instrument), X – number of colonies (CFU) at 1000 L (1 m^3) of air.

The microbial flora from the walls was detected using the Count-Tact applicator and the plate Count-Tact (BioMerieux). Classification of isolated fungi was made with an accordance to the current procedures. After incubation at 27°C for three days quantitative analysis and morphological evaluation of fungal colonies were carried out, and depending on the nature of the fungi cultures the plates were incubated for up to 14 days to allow identification. The raw counts of colonies on the agar plates were adjusted by reference to statistical scaling tables applying to the particular sampler. The cultured fungi were identified from macroscopic and microscopic characteristics, and biochemical tests were appropriate. Yeast-like fungi were identified by means of original Candida ID (bioMerieux) medium, API 20C AUX (bioMerieux) identification sequence as well as CandiSelect (Bio-Rad) medium. For moulds, microscopical evaluation of morphological elements in preparations was performed. Laboratory studies were performed in Poland. Temperature and humidity were measured by thermo-hygrometer PWT-401 (Elmetron).

Wilocoxon's paired test was used. Significance was defined as a p value of ≤ 0.05. This analysis was performed on a personal computer with a commercially available statistics program (Statistica 6.0).

RESULTS

Tab. 1 presents of fungal occurrence in the air of rooms of the Department of Pulmonology in the Kavala Hospital in Greece. The following fungal pathogens isolated from air were: *Asperegillus species, Candida albicans, Candida spp., Penicillium species* and *Acremonium species*. Similar isolates from the walls of the Department Pulmonology were found. Numbers of airborne culturable fungi were highest in the storing room and corridor. Similarly, the highest number of air borne fungi were isolated from wall of the corridor and the table in the social room. Mean number of fungi colonies isolated from air was 55.75 ±47.50, mean temperature 24.97 ±0.19°C and humidity 62.21 ±4.60. No significant correlation (p=0.234) between CFU of fungi in air and temperature was noted. Similarly, no relationship between CFU of fungi in air and humidity was found.

Tab. 2 presents of fungal occurrence in the air of rooms of the Department of Internal Medicine and Chemotherapy in the Kavala Hospital. The following fungal pathogens isolated from air were: *Candida albicans, Candida albicans spp.,* and *Asperegillus species*. No *Penicillium* and *Acremonium species* were detected in air of this Department. In contrast only *Candida species* were isolates from the walls. Mean number of fungi colonies isolated from air was 17.80 ± 10.98, mean temperature 26.18 ±0.36°C and humidity 60.16 ±0.69. No significant correlation (p=0.183) between CFU of fungi in air and temperature was noted. Similarly, no relationship between CFU of fungi in air and humidity was found. Significant difference ($p<0.001$) in mean number (55.75 ± 47.50 vs 17.80 ± 10.98) of fungi colonies between the Departments was found.

DISCUSSION

In the present study, we demonstrated considerable numbers of fungi in the air of the two Departments of Kavala Hospital in Greece. This is the first study carried out in Kavala Hospital comparing the fungal contamination of air in the Department of Pulmonology and Department of Internal Medicine. We performed the study in May. In the literature there is clear evidence of seasonal differences in the numbers of fungi in indoor air. For example, Lumpkins et al. [4] reported that numbers were higher in summer and autumn than in spring and winter. In our previous report [7] in the Neonatal Department and Intensive Care Unit and Palliative Care in the same hospital in Kavala we detected the following fungal pathogens

Table 1. **Fungal occurrence in the air of rooms and walls of the Department of Pulmonology in the Kavala Hospital in Greece.**

DEPARTMENT OF PULMONOLOGY II								
Air						Wall		
Site of sampling	Number of colony	Taxonomy	Temp.	Humidity	Air flow	Site of sampling	Number of colony	Taxonomy
Bathroom	6	6 Candida albicans	25.6	64	0.04	Room 315	8	5 Candida albicans 3 Candida sp.
Corridor	10	8 Candida albicans 2 Candida sp.	25.5	65.9	0.01	Bathroom	28	23 Candida albicans 5 Candida sp.
Room 318	34	25 Candida albicans 9 Candida sp.	25	65.1	0.02	Room 318	100	Penicillium sp.
Room 315	1	Acremonium sp.	24.2	67.1	0.02	Corridor	240	Acremonium sp.
	12.75 ±14.20		25.07 ± 0.63	65.52 ± 1.30	0.02 ± 0.02		94.0 ± 105	
DEPARTMENT OF PULMONOLOGY I								
Bathroom	16	15 Candida albicans 1 Candida sp.	25.7	62.4	0.08	Room 301	200	Penicillium sp.
Corridor	250	Aspergillus sp. Candida sp.	25.6	65.7	0.01	Social room	6	6 Candida albicans
Room 309	31	27 Candida albicans 4 Candida sp.	25.7	62.4	0.08	Table in social room	250	Candida sp. Penicillium sp.
Room 301	29	2 Penicillium sp. 17 Candida albicans 10 Candida sp.	26.2	65	0.01	Dirty room	10	10 Candida albicans
Storing room	300	Aspergillus sp. Candida sp.	26.5	65.4	0.14	Corridor	7	7 Candida albicans
Social room	16	2 Penicliium sp. 14 Candida albicans	25.7	64	0.01	Room 309	11	11 Candida albicans
	107 ± 131.07		25.09 ± 0.37	64.15 ± 1.30	0.055 ± 0.053		80.66 ± 112.6	
BRONCHOSCOPY								
Room II	90	50 Aspergillus sp. 30 Penicillium sp. 10 Candida albicans	24.4	56.1	0.01	Room I	20	18 Candida albicans 2 Candida sp.
Room I	5	5 Candida albicans	25.1	57.8	0.01	Bronchoscope	300	Candida sp.
						Over sink	12	12 Candida albicans
	47.5 ±60.10		24.75 ± 0.49	56.95 ± 1.22	0.01 ± 0		110.67±164.01	

Table 2. **Fungal occurrence in the air of rooms and walls of the Department of Internal Medicine in the Kavala Hospital in Greece.**

DEPARTMENT OF INTERNAL MEDICINE								
Air	Wall							
Site of sampling	Number of colony	Taxonomy	Temp.	Humidity	Air flow	Site of sampling	Number of colony	Taxonomy
Room 212	32	1 Aspergillus sp. 26 Candida albicans 5 Candida sp.	26.8	59.9	0.02	Room 211	7	7 Candida albicans
Room 211	8	8 Candida albicans	26.2	60.9	0.01	Room 212	12	10 Candida albicans 2 Candida sp.
Corridor	27	22 Candida albicans 5 Candida sp.	26	60.9	0.01	Corridor	10	10 Candida albicans
	22.23 ± 12.66		26.33 ± 0.41	60.56 ± 0.57	0.013 ± 0.005		9.66 ± 2.51	
DEPARTMENT OF CHEMOTHERAPY								
Room	13	1 Aspergillus sp. 12 Candida albicans	26	59.4	0.01	Room	4	3 Candida albicans 1 Candida sp.
Corridor	9	1 Aspergillus sp. 8 Candida albicans	25.9	59.7	0.01	Corridor	3	3 Candida albicans
	11.0 ± 2.82		25.96 ±0.07	59.55 ± 0.21	0.01 ± 0		3.5± 0.70	

isolated from air *Candida albicans*, non-*Candida albicans*, *Penicillium species Acremonium*, *Rhodotorula species*, and *Aspergillus species*. *Candida albicans* and *Penicillium species* were dominated fungi in the air of Neonatal Department and Intensive Care Unit.

In contrast, in the present study we found significant number of *Aspergillus species* and *Candida albicans*. The environmental fungal load of three hospitals was studied in representative regions in Greece (Thessalonika, Northern Greece, Athens, Central Greece and Heraklion, Southern Greece) [8]. Air, surfaces and tap water from high-risk departments were sampled monthly during one year. Air fungal load was lower in winter and higher in summer and autumn but seldom above acceptable levels. *Aspergillus species* constituted 70.5% of the filamentous fungi isolated. *Aspergillus niger* was the most prevalent species in the air of all the hospitals followed by *Aspergillus flavus* and *Aspergillus fumigatus*. The least conta- minated departments were the intensive care units, whilst most contaminated were the solid organ transplantation in Athens and haematology departments in Thessalonika. Our findings are accordance with these findings, in the present study we also detected high number of *Aspergillus species*. No correlation between fungal species, season, hospital or departments was observed. The presence of *Aspergillus*, may pose a potential threat to the health of patients of these rooms [9-11]. Fungi in these and other genera affect humans in complex ways and are capable of causing a variety of diseases, such as infection, allergy and irritation, and toxicosis. Exposure to fungi has been unequivocally associated with exacerbation of asthma. Fungi, especially moulds, can cause devastating infections in these high-risk patients [11-13]. Moreover, surface contamination with settled fungal spores, which is not detected by air sampling, could also present a source of potential colonization [11]. Fungal conidia enter buildings through windows, doors or ventilation systems and sediment onto surfaces, survive in dust or grow on organic matter present in materials such as ceiling tiles [14,15]. In the hospital setting, construction work that liberates large amounts of *Aspergillus* spores has been identified as the source of nosocomial aspergillosis.

Gioulekas et al. [16] created a database on fungi spores circulated and investigated skin sensitivity of asthmatics by using skin prick tests. Daily records and identification of 15 airborne fungi spores species were conducted, using a Burkard trap during 1987-2001. Skin sensitivity to five most common fungi spores extracts was investigated, by using skin prick tests in a total of 1311 asthmatics with atopy, submitted to the Out-Patient Clinic of Asthma (Pulmonary Department in Thessaloniki) in 1990-2001. The fungi spores recorded in the 15-year period were as follows: *Cladosporium spp*. (72.2%), *Alternaria spp*. (9.8%), *Ustilago spp.* (8.1%), *Ascospores* (2.7%), *Agrocybe spp.* (1.5%), *Helminthosporium spp.* (1.4%), *Leptosphaeria spp* (1.2%), *Agrogybe spp.* (1.1%), whereas the species *Botrytis, Stemphylium, Pleospora, Nigrospora, Epicoccum. Fusarium, Torula* and *Phoma* presented concentrations <1%. The highest numbers of airborne fungi spores were recorded during summer. Positive skin reaction to fungi spores was observed in 421 (32%) patients of the 1311 asthmatics. Positive skin reaction to *Altemaria* species was observed in 177 patients (13.5%), in 98 (7.4%) to *Cladosporium*, 65 (5%) to *Aspergillus*, 45 (3.4%) to *Fusarium* and 36 (2.7%) to *Rhizopus*. Authors concluded that patients were more sensitive for the species of *Alternaria, Cladosporium* and *Aspergillus*.

In contrast, in our study we found a high occurrence of *Aspergillus species* and *Candida albicans* in the air in the both tested departments.

CONCLUSIONS

In conclusion, the main fungal pathogens isolated from the air samples were *Aspergillus* and *Candida albicans*. Fungal occurrence in the air of rooms and walls varied between the both departments of the same hospital. The opportunistic fungi can be inhaled in the hospital by patients and exacerbate asthmatic attacks and pneumonia. Further investigations on isolation of the fungal pathogenes from the air samples of these Departments are needed.

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