

Concentrations of Airborne Fungal Contamination in the Medical Surgery Operation Theaters (OT) of Different Hospitals in Northern Jordan

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Abstract

Total count and diversity of the airborne yeasts and filamentous fungi in the medical surgery operation theatres (OT) of six hospitals in northern Jordan were investigated. Sixty five air samples of 100 liters volume/min were collected by a microbiological air sampler from these units during the period June-December/2005. Air samples were impacted on Sabouraud Dextrose Agar (SDA), and then incubated at 25 °C for 21 days. All fungal colonies appeared on agar plates were sub-cultured on two SDA plates, after that incubated at 37 °C and 25 °C for 48 h and 21 days, respectively, and then identified based on the microscopic colony morphology and germ tube test. The average fungal count ranged between 88 and 259 CFU/m³ with the highest count observed in hospital E (259 CFU/m³), and the lowest in hospital A (31 CFU/m³). More diverse colonies (2-13) were observed during June-September than after September (2-3) with the highest diversity (13 colonies) in hospital E. *Aspergillus* spp. (*A. fumigatus*, *A. niger*, *A. flavus*, *A. glaucus* and *A. terreus*) and other molds (*Penicillium* spp., *Mucor* spp., *Rhizopus* spp., *Graphium* spp., *Geotrichum* spp., *Trichophyton* spp., *Scopulariopsis* spp., *Fusarium* spp and *Microsporum* spp) were identified. Two types of yeasts were identified as *Candida* spp. and *Blastomyces* spp.

المخلص

تم في هذه الدراسة تحديد العدد الكلي وتنوع الفطريات و الخمائر المنقولة بواسطة الهواء في غرف العمليات لستة مستشفيات في مدينة اربد. جمعت 65 عينة هواء (100 لتر/دقيقة) بواسطة جهاز خاص بذلك للفترة مابين شهر حزيران إلى شهر كانون الثاني/2005، وذلك باستخدام أطباق الوسط الزراعي SDA والتي حفظت على درجة حرارة 25 مئوية لمدة 21 يوم. تم إعادة تنمية كل مستعمرة فطرية ظهرت على هذه الأطباق على طبقين من وسط SDA وحفظها على درجة حرارة 37 و 25 مئوي لمدة يومين و 21 يوم على التوالي ومن ثم شخّصت هذه الفطريات بناء على الشكل المجهرى وفحص أنبوب الإنماء. تراوح عدد الفطريات ما بين 88-259 CFU/m³ وبملاحظة أعلى عدد في مستشفى E (259 CFU/m³) وأقل عدد في مستشفى A (31 CFU/m³). كان التنوع أكثر في أشهر حزيران-أيلول (2-13) عما عليه في بقية مدة الدراسة (2-3) وملاحظة أكثر تنوعا (13 مستعمرة) في مستشفى E. تم عزل وتشخيص العديد من الفطريات مثل (*Aspergillus* spp. (*A. fumigatus*, *A. niger*, *A. flavus*, *A. glaucus*, *A. terreus*) وفطريات أخرى مثل (*Penicillium* spp., *Mucor* spp., *Rhizopus* spp., *Graphium* spp., *Geotrichum* spp., *Trichophyton* spp., *Scopulariopsis* spp., *Fusarium* spp and *Microsporum* spp) و نوعين من الخمائر هي *Candida* spp. و *Blastomyces* spp.

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KeyWords: Airborne; Fungi; Jordan; Hospitals; Operating Theaters.

1. Introduction

The presence of high concentrations of airborne microorganisms within the indoor environments is of increasing concern with respect to many acute diseases, infections, and allergies (Lugauska and Krikstaponis, 2004), and it is an indication of degree of cleanliness of these environments. Indoor environment, of which hospitals is of particular concern, contains different types of microorganisms (Saad, 2003), thus patients may serve

as a source of pathogenic microbes to other patients, staff, and hospital visitors.

Bio-aerosols, of which fungal spores are one of the major types of microorganisms, can be present in all hospital environments, and may be transmitted through air, outdoor air, visitors, patients, and air conditions (Beggs, 2003; Manuel and Kibbler, 1998). The evaluation of bacterial count, types, and diversity in hospitals rooms, especially in sensitive units like medical surgery operation theatres (OT) has raised worldwide concern. Approximately 10% of all patient infections are suspected

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to be hospital-acquired (Meers et al., 1990). These infections can have serious consequences in terms of increased patient mortality, morbidity, and length of hospital stay and overall costs.

This study investigates the measurements of airborne concentrations of fungi in the medical surgery operation theaters (OT) of six different hospitals in northern Jordan. Isolation and identification of airborne fungal genera and/or species being impacted from the indoor environment of OT of these hospitals are reported.

2. Materials and Methods

2.1. Hospitals Visited

The study was carried out looking for airborne fungi in the medical surgery operation theatres (OT) for six different hospitals serving a population of about 1.3 million in Irbid and its providence, Northern Jordan (Table 1).

Table 1: Numbers and diversity of isolated fungi/m³ during air samplings in the atmosphere at the medical surgery operation theaters (OT) of six different hospitals in Northern Jordan during June-August and September to December.

Hospital			First period of study (June to August)		
	No. of beds	Date Established	No. of Air Samples	Range No. of Colonies/Diversity of Colonies	Average No. of Colonies/Diversity of Colonies
A	850	2002	10	0-80/0-4	31 ^{*,**} /2
B	150	1985	6	45-340/4-12	137 [*] /9
C	100	2000	3	0-180/0-5	84 ^{***} /3
D	100	1992	7	15-70/2-5	43 ^{***} /4
E	350	1973	10	70-720/5-22	259 ^{***} /13
F	150	1988	4	5-60/1-6	38 [*] /4
Total No. of Air Samples			40		
Total No. of Colonies (%)			112 (42.1%)		
% of <i>C. albicans</i> from total isolates			3.6		
Hospital			Second period of study (September to December)		
	No. of beds	Date Established	No. of Air Samples	Range No. of Colonies/Diversity of Colonies	Average No. of Colonies/Diversity of Colonies
A	850	2002	4	40-110/1-4	80 [*] /2
B	150	1985	3	50-100/2	80 [*] /2
C	100	2000	4	30-180/1-4	118 [*] /3
D	100	1992	4	40-200/2-6	103 [*] /3
E	350	1973	6	90-220/2-4	133 [*] /3
F	150	1988	4	100-210/2-3	148 [*] /2
Total No. of Air Samples			25		
Total No. of Colonies (%)			154 (57.9%)		
% of <i>C. albicans</i> from total isolates			2.0		

* Denote no significant differences between hospitals

** Denote significant differences between hospitals when hospital E is compared to A, C and D hospitals ($P \leq 0.05$)

2.2. Air Sampling

Between June and December (2005), a total of 65 air samplings were performed in the internal atmosphere of OT units. Each air sampling was performed using a special device; Microbiological Air Sampler (M.A.Q.S.II-90)/OXOID, UK). That device can hold 90 mm Petri dishes containing sabouraud dextrose agar (SDA) within an autoclavable anodized aluminum head of 380 holes. The sampler was set at an air-sampling rate of 100 l/min for two minutes per sample. During each visit, duplicate air samples were collected with sampling made at one meter elevation from the OT floor (i.e. at the same level of the patient's bed). The anodized aluminum head of the microbiological air sampler between the duplicate air samples collection was sterilized by 70% alcohol using a

sterile cotton swab. All samplings were made when the OT was not in use, i.e. at a period of idling for next operation.

2.3. Sample Processing

After impacting the air borne microbial samples on SDA (Oxoid, UK), they were transported to laboratory and immediately incubated at 25° C with daily observation of the plates for fungal growth up to 21 days. Counts of different fungal growths were coded as they appeared on SDA plates and were designated to their specific genus and species, and later were recorded by using the colony counter (560, Suntext, Labolan).

2.4. Fungal Identification

During incubation period, different fungal colonies were subjected to macroscopic and microscopic examination to observe their growth, nature of their

mycelium, and hyphae structure. Filamentous fungal growth - as mold and /or yeast- were present on SDA media plates; and were sub-cultured on two separate SDA culture plates. One plate was incubated at 37° C, and the second was incubated at 25° C (Koneman *et al.*, 1997). Pure culture growth of each mold and/or yeast colony appeared on those plates; and was examined under magnification for their microscopic structures and cross identified, by using mycological keys manuals and textbooks. Direct wet mounts in lacto-phenol cotton blue (LPCB) and microculture slides preparation were made to determine the nature of the fungal hyphae and the fructifications, such as conidiophores, conidia production. And the kind of conidia produced by the molds was loosely examined.

Yeast colonies were determined whether being colonies of *Candida albicans* or not, according to procedure outlined by Larone (1987). This test was performed by transferring a loop-full from single colony growth of yeast on the SGA plates to 0.5 ml of sterile serum in a test tube; and incubated the tubes at 35-37°C for approximately three hours. The yeast-serum culture examined as wet mount under magnification (X400) for the formation of germ tube (psuedohyphae) which is an indication of positive identification of *Candida albicans* (Larone, 1987).

2.5. Statistical Analysis

Analyses of variance for all data were performed using statistical analysis system (SAS Institute Inc., 2000). Means were separated by the least significant differences (LSD) at $\alpha = 0.05$.

3. Results

From June to December-2005, a total of 65 air samplings were made from the atmosphere of medical surgery operation theaters (OT) of six hospitals in Irbid city, Northern Jordan (Table 1). A total of 266 fungal colonies were isolated and recognized as 112 colonies (42.1%) appeared during June to August (Table 1). Molds comprised 45.5% while yeasts comprised 54.5% (data not shown). However, 154 colonies (57.9%) (Table 1) were recognized during September to December of which 41% were molds and 59% were yeasts (data not shown).

Fungal counts in OT of the first period of study ranged between 31 and 259 CFU/m³. However, fungal counts in the second period of study ranged between 80 and 148 CFU/m³. Data showed that fungal counts in OT during the second period were higher than the first period with the exception of hospitals B and E (Table 1). This variation was not clear in hospitals A and C and during the same periods. When the fungal count in the different hospitals was compared between the two periods, data indicated that hospitals C, D and F exhibited higher counts during Autumn than Summer (Table 1). In general there is a significant difference in total fungal count between hospitals E and A, B, D and F hospitals ($P < 0.05$).

Fungal diversity in the first period of study ranged between 2 and 13 different colonies. However, fungal diversity in the second period of study ranged between 2 and 3 different colonies. Data revealed that fungal diversity in the OT during Summer was higher than Autumn and particularly in hospitals B and E (Table 1).

This variation was not clear in hospitals A and C and during the same periods.

During both periods, filamentous fungi were distributed at considerable levels in all visited hospitals with variation among different species of isolated fungi. *Aspergilla*; such as *A. fumigatus*, *A. niger*, *A. flavus*, *A. glaucus* and *A. terreus* constituted 78-85% / 71-80% of the isolated fungi (data not shown). The other proportion (15-22% / 20-29%) of isolated molds was identified as *Penicillium* spp, *Mucor*, *Rhizopus*, *Graphium*, *Geotrichum*, *Trichophyton*, *Scopulariopsis*, *Fusarium* and *Microsporium* (data not shown).

Distribution of airborne yeasts was different during the two periods of study with *Candida* spp. (76%) and *Blastomyces* spp. (15%) being recognized during June to August (data not shown). However, during September to December for the above yeasts, this distribution was 78% and 4%, respectively (data not shown). *Candida albicans* encountered 3.6% and 2% of total fungal isolates during Summer and Autumn, respectively (Table 1).

4. Discussion

Hospital environments are complex environments because they contain different types of microorganisms. Airborne microorganisms are one of these microbes and their presence, numbers, and types can indicate the degree of cleanliness of these environments. There are wide varieties of factors which influence airborne counts, and therefore influence hospital infection rates (Jaffal *et al.*, 1997; WHO, 2002).

Fungi and bacteria are the major types of microorganisms present in all hospital environments that may be transmitted through air, outdoor air, visitors, patients, and air conditions. These are the major sources of hospitals indoor contamination (Beggs, 2003; Manuel and Kibbler, 1998). The level and diversity of biocontamination in hospitals environments depend on different factors such as the number and activities of visitors, patient, design of hospitals rooms, disinfectant process and methods, outdoor air and dust, and other factors (Sessa *et al.*, 2002; Saad, 2003). The evaluation of count, types, and diversity of biocontamination in hospitals rooms especially OT is very important to control and prevent hospital acquired infections (HAI).

Results showed that fungal counts in all studied hospitals units during Autumn are higher than during Summer. The higher number of fungi in hospitals in these seasons may be related to occupant density, temperature, and level of humidity. In addition, the bioaerosols containing microorganisms may reside in Autumn for long time in air than Summer. These results are consistent with those reported by Hou and Li (2003).

The fungal counts in OT of hospital E were higher in Summer than in Autumn, because the samples were collected after the process of disinfection and sterilization of these units in this hospital. The quantitative study of OT showed that the number of microorganisms in OT was considerably low. This is due to the high sanitary standards in OT, as compared to other hospital areas. The old design of hospitals B, D and E in comparison with hospital A that has been established in 2002 is the major reasons of the high level contamination. However the high level of

contamination in hospital F may be due to outdoor contamination.

Diversity of fungi is usually related to the count (Spengler and Saxton, 1983; Flannigan, 1992). Data showed that more fungi were isolated in Autumn than in Summer. The results are consistent with Hou and Li (2003) and Klanova and Hollerova (2003).

Studying airborne fungal spores is important to understand dissemination, spread, and movement of the microbes, particularly the pathogenic ones in the atmosphere (Moustafa and Kamel, 1976). During Autumn, the percentage of yeast increased while mold decreased in Summer. These results may be correlated with high level of humidity in Autumn than in Summer (Beggs, 2003). The common genera of fungi that are frequently isolated from the hospitals air are *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Graphium*, *Geotrichum*, *Trichophyton*, *Scopulariopsis*, *Fusarium* and *Microsporium* spp. However, the common genera of yeasts that are frequently isolated from hospitals are *Candida* spp. and *Blastomyces* spp. (Lugauska and Krikstaponis, 2004). Significant numbers of *Aspergillus* spp. (71-85%) and *C. albicans* (35.5-37%) were shown in comparison with other fungal species. *Aspergillus* spp. and *C. albicans* as reported in different studies (Ahmad et al., 2003; Weinberger et al., 1997; Overberger et al. 1995; Harvey and Hyers, 1987); and were considered as the major source of hospital fungal infections. Manuel and Kibbler (1998). Overberger et al. (1995) found that 70-80% of the fungi in hospitals air were *Aspergillus* spp. This study showed similar distribution of the mold and yeast species.

5. Conclusion

In conclusion, airborne concentrations of fungi in the operating theaters (OT) of six different hospitals in northern Jordan indicated higher counts but less diversity during autumn than during summer. The high sanitary standards in OT units and new design of hospitals are the major reasons of the low level of contamination.

Acknowledgments

Deanship of Scientific Research at Jordan University of Science and Technology funded this research (Grant No. 37/2005).

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