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Full Length Research Paper

Assessment of airborne pathogens in healthcare settings

K. Qudiesat[1], K. Abu-Elteen[2]*, A. Elkarmi[2], M. Hamad[3] and M. Abussaud[4]

[1]Jordan University Hospital, Amman, Jordan.
[2]Department of Biology and Biotechnology, The Hashemite University, Zarqa, Jordan.
[3]School of Medicine, Taif University, Taif, Saudi Arabia.
[4]Department of Biological Sciences, Yarmouk University, Irbid, Jordan

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An investigation of the air quality and quantity of airborne microbes in selected hospitals of Zarqa city, Jordan, was carried out to assess the level of airborne pathogens and to establish standards for further reference. Using a microbial air sampler, air samples were taken from a governmental and a private hospital in Zarqa city. Three factors were investigated to determine how these factors affect the microbial counts, namely the kind of hospital, the type of room and the time of sampling. Nine bacterial species were identified. In a governmental hospital, *Staphylococcus aureus* (16.2%) was found to be the most common organism, followed by *Micrococcus luteus* (13.3%) and coagulase-negative *Staphylococcus* (13%). Coagulase-negative *Staphylococcus* (17.2%), followed by *S. aureus* (16.8%) and *M. luteus* (10.7%) were found to be the most common in a private hospital. *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. and *Alternaria* spp. were isolated in both hospitals. The indoor air of the governmental hospital was more contaminated than that of the private hospital in all units. Maximum bacterial rates were detected in the patient rooms, while minimum bacterial rates were detected in the operating rooms and neonatal wards. The time of visit showed higher microbial rates in governmental hospital, while the private hospital was not affected by this factor. Microbial rates in the patient room, main entrance and intensive care unit (ICU) were found to be influenced by the time of sampling, while the operating room and neonatal ward were not. Several explanations might be involved in these variations, that is, the age of hospital building, the number of beds, the number of visitors, disinfection procedures and ventilation systems. We concluded that the indoor air quality of hospitals in Zarqa city, especially the governmental hospital, needs more care and surveillance and should be given priority in Jordan.

Key words: Air sampling, airborne bacteria and fungi, indoor air, hospital, Jordan.

INTRODUCTION

Hospitals and other healthcare facilities are complex environments that require ventilation for comfort of patients and control of hazardous emissions (Chuaybamroong et al., 2008; McCarthy et al., 2000). Moreover, the biological quality of air in hospital environments is of particular concern as patients may serve as a source of pathogenic microorganisms to staff and hospital visitors, in addition to fellow patients (Obbard and Fang, 2003). Although hospitalization and medical procedures are designed to cure diseases, they can sometimes inadvertently introduce pathogenic microorganisms into the body and initiate a nosocomial infection (NI) (Atlas, 1995).

The most important source of airborne pathogens inside the hospital is the infected patient (Hambraeus, 1988). Airborne transmission occurs when pathogenic microorganisms are transferred from an infected to a susceptible individual via the air (Atlas, 1995). The predo-minant mechanism that makes the pathogens airborne is the production of aerosol droplets by sneezing or coughing, and their subsequent loss of water which allows them to float in the air over considerable distances and for a long

*Corresponding author. E-mail: salma@hu.edu.jo. Tel: (+ 962) 53903333. Fax: (+962) 53903349

time (Emmerson, 1995). Biological aerosols contain bacteria, viruses, yeasts, molds and fungal spores (Gillette, 2000; Nevalainen et al., 1993). Under special clinical circumstances, skin lesions may also be a source of airborne particles (Hambraeus, 1988).

In spite of environmental conditions, e.g. dryness, temperatures and ultraviolet radiation, which may prevent microorganisms from growing in unfavourable environments, they still reach new hosts through the air. Some bacteria, particularly Gram-positive bacteria such as *Streptococcus pneumoniae and Staphylococcus aureus*, can survive for several months in dust particles. Also, fungal spores and viruses can survive for longer periods of time. The incidence of airborne infections has increased in recent years, because many new buildings are sealed and have self-contained circulating air systems for temperature control (Atlas, 1995; Augustowska and Dutkiewicz, 2006; Matar et al., 2005).

Controlling airborne pathogens in healthcare facilities is not only important for the safety of the patient, but it is also important for hospital personnel. Various contamination control procedures can limit exposure and risk of infection (Montz and Edward, 2000). Although it is not possible to eliminate all NI, their incidence can be significantly reduced by implementation of appropriate infection control policies (Abussaud, 1991). There is a demand to reduce airborne microorganisms and their fall out (the bioburden of microorganisms causing infection in healthcare facilities). Furthermore, it is important to identify and accumulate bioburden data of these facilities where the maintenance of a clean environment and the accumulation of data on airborne microorganisms is required (Shintani et al., 2004; Li and Hou, 2003). The counting and identification of microbes in air is not an easy task. Various methods are used and these can be divided into four groups: counts of colony forming units per cubic meter of air (CFU/m3); counts of CFU on settle plates; counts under a microscope; and measurement of a chemical component of the microbial cells per cubic meter of air (Pasquarella et al., 2000). There is no single method of choice for sampling airborne loads (Dharan and Pittet, 2002; Jaffal et al., 1997; Shintany et al., 2004; Wu et al., 2000). However, impactor air samplers are the most widely used for the quantification of contamination (Nesa et al., 2001; Morris et al., 2000). Their advantage lies in the fact that agar plates can be incubated without further treatment, which means that colonies grow directly from collected viable airborne particles (Gangneux et al., 2006; Morris et al., 2000; Prigione et al., 2004).

Hospital aerosols must be regularly investigated. Gröschel (1980) reported that sampling of air may be performed in hospitals for several purposes, e.g. epidemiologic, surveillance, research, safety or quality control purposes. Other studies have reported that occupant density is a key factor affecting concentrations of airborne bacteria, and humidity is also important depending on the particular location within the hospital Obbard and Fang, (2003). Li and Hou (2003) have concluded that the significant particle concentration fluctuations in operating rooms may be related to variations in operating personnel numbers and activities.

Goodley et al. (1994) have reported on Aspergillus infections, primarily invasive pulmonary aspergillosis. Building works carried out in the vicinity of ward areas can generate large aerosols of infective particles. Nevertheless there was no evidence of gross seasonal variation and it would appear that climatic conditions did not influence spore counts of A. fumigatus in the air. Several authors have concluded that mold spores may enter the hospital through windows or inadequate air filtration systems (Gerson et al., 1994; McCarthy et al., 2000; Pastuszka et al., 2005). Surfaces, such as carpets, potted plants and multiple-hole false ceilings are potential sources of fungal contamination. Dust might accumulate in these areas and spores may enter the patient room as contaminants on personnel's clothing.

In Jordan, an infection control committee or program is not mandatory in hospitals and does not exist in most of them (Khuri-Bulos et al., 1999). Therefore, this study was conducted to gain knowledge regarding the air quality and the quantity of airborne pathogens in the indoor air of two selected Jordanian hospitals in Zarqa city. The data can be used to set standards for levels of acceptable microbial population and can also be used to suggest suitable guidelines in order to decrease the microbial rates in indoor air.

MATERIALS AND METHODS

Sampling sites

For this study, two hospitals were selected from the Zarqa governorate, which has more than one million two hundred thousand inhabitants. The hospitals were the only governmental hospital (built in 1960, 294 beds) and a private hospital (one of three hospitals built in 1986, 80 beds). Air samples (500 L air/sample) were taken from the following sites in both of the hospitals: intensive care unit (ICU), operating room (OR), neonatal ward (NW), the main entrance of the hospital (ME), and patient room (PR). At each location, three air samples were taken at three different time periods (10:00 - 12:00 am, 2:00 - 4:00 pm and 7:00 - 9:00 pm). In addition, three triplicates of surface swabs were taken from the operation rooms and neonatal wards, the air conditioning systems, the ventilation grills in the intensive care units and patient rooms. All samples were taken during December 2005.

Air sampling

A microbial air sampler (PBI International, Milano, Italy) was used for sampling of airborne bacteria and fungi. The microbial air sampler was operated at an air flow-rate of 100 L/min. The sampling time was 5 min to avoid drying of the agar surface and overloading of the collection plate (Stetzenbach et al., 2004). The total volume of air that was aspirated onto an agar plate was 500 L in each sample from each location (room). The air sampler was set up at a height representative of the normal human breathing zone, that is, 1.5 m above floor level (Obbard and Fang, 2003). Between measurements the sampler was cleaned by swabbing with 70%

Table 1. Enumeration of bacteria (CFU/m^3 air) according to the kind of hospital, the type of room and the time of sampling.

		Bacterial CFU/m^3 air		
		Morning (n = 3) 10 - 12 am	Afternoon (n = 3) 2 - 4pm	Evening (n = 3) 7 - 9 pm
Governmental Hospital	ICU	149	197	147
	OR	79	107	93
	NW	95	82	69
	ME	174	229	163
	PR	198	254	185
Private Hospital	ICU	109	107	121
	OR	34	25	29
	NW	33	46	27
	ME	120	115	87
	PR	145	163	137

ICU: Intensive Care Unit, OR: Operating Room, NW: Neonatal Ward, ME: Main Entrance, PR: Patient Room. LSD p-values from comparison of governmental and private hospitals according to type of room (ICU p <0.000, or p<0.000, NW p<0.000, ME p<0.000 and PR p<0.000)

ethanol (Wu et al., 2000).

Culture media and microbial identification

Nutrient agar (NA) (HiMedia Laboratories Limited, Mumbai, India) supplemented with 100 mg/L cyclohex-amide was used for the sampling and cultivation of bacteria (Obbard and Fang, 2003). For isolation of fungi, Sabouraud dextrose agar (SDA) (HiMedia) supplemented with 10 mg/L chloramphenicol was used (Rainer et al., 2000). Three replica plates of each medium were used for the isolation of bacteria and fungi. Nutrient agar plates were incubated at 37°C for 48 h to allow the growth of aerobic bacteria, while SDA plates were incubated for up to 5 days at 25°C to allow the growth of fungal colonies.

Bacterial colonies were initially characterized by morphology and microscopic appearance, and identified further by biochemical tests. These tests included catalase, coagulase, indole, methyl-red and Voges-Proskauer, fermentation of glucose, lactose, and mannitol, citrate utilization, gelatin hydrolysis, and starch hydrolysis. Blood agar, MacConkey agar, mannitol salt agar, eosin-methylene blue agar and Muller Hinton agar were used for differentiation. The biochemical and physiological characteristics of identified bacterial species were performed according to Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984; Sneath et al., 1986). Confirmation of the identity of the bacterial species was carried by Jordan University Hospital, using other methods such as API 20STAPH, API 20E and API 20NF (Abu-Elteen et al., 2000; Jaffal et al., 1997).

A wet mount preparation of each fungal colony was prepared by using Lactophenol-cotton-blue solution and examined microscopically. Identification of fungi was based mainly on growth colonial appearance, microscopic examination of the spore and hyphal characteristics of the stained preparations (Frey et al., 1979; Larone, 1995; Samson et al., 2002).

Statistical analysis

The total number of colony forming units (CFU) was enumerated and converted to organisms per cubic meter of air (CFU/m3). The mean of the triplicate samples of each microorganism (bacteria and fungi) was calculated in all sample locations at both governmental and private hospitals. The data were processed with STATISTICA 5.0 (StaSoft, USA and statistical significant differences were determined by one-way and two-way analysis of variance (ANOVA), and the Fisher's Least Significant Different (LSD) test was also used. A p-value less than 0.05 were considered statistically significant.

RESULTS

Air samples from each sampled unit were taken and used for enumeration and isolation of airborne bacteria on NA plates, and for the enumeration and isolation of airborne fungi on SDA plates.

Enumeration of bacterial colonies from air samples

The bacterial counts on NA (CFU/m3 air) ranged from 25 CFU/m3 air, which was isolated from the operating room of the private hospital, to 254 CFU/m3 air from the patient room of the governmental hospital (Table 1). The bacterial CFU/m3 air in the governmental hospital was significantly higher than that in the private hospital in all five units. In both hospitals, the patient rooms had the maximum bacterial rates, and the minimum rates were detected in the neonatal wards and operation rooms (Figure 1A).

Enumeration of fungal colonies from air samples

The fungal counts (CFU/m3 air) on SDA ranged from 2 CFU/m3 air, which was isolated from the operating room of the private hospital, to 73 CFU/m3 air from the main entrance of the governmental hospital (Table 2). The fungal CFU/m3 air of the governmental hospital was higher than that of the private hospital in all units, except

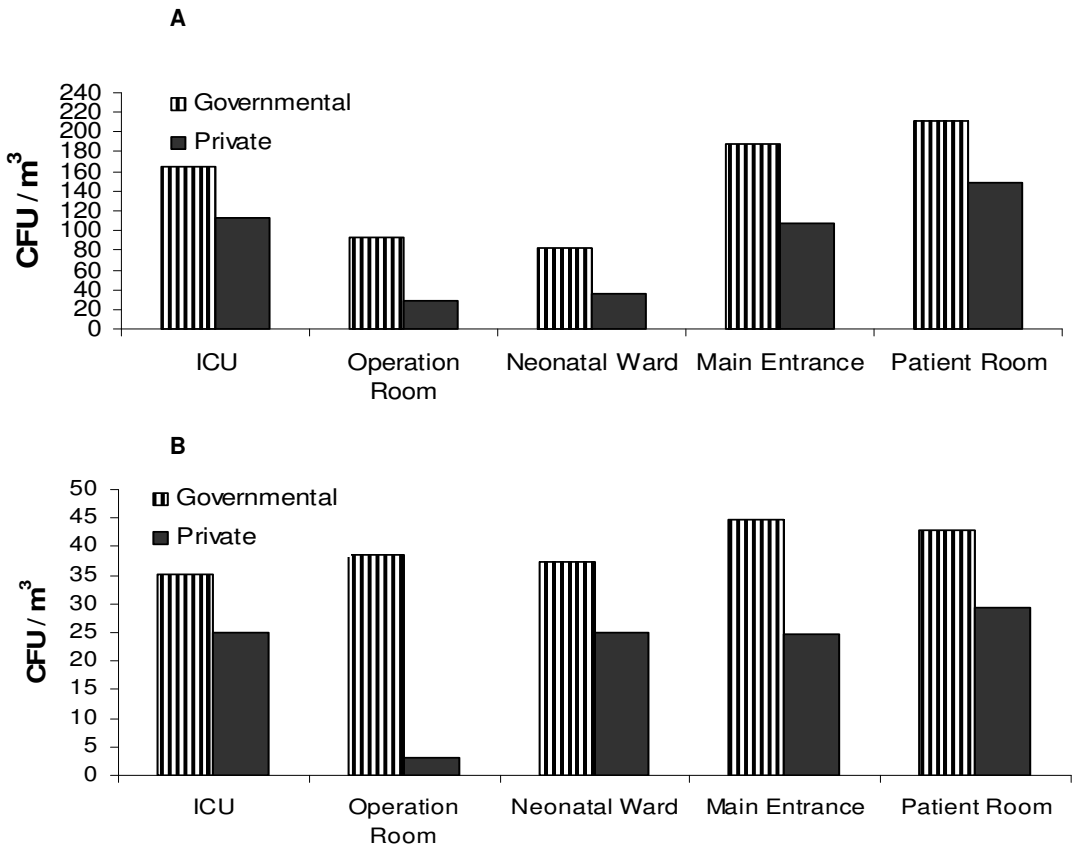

Figure 1. The effect of the kind of hospital and the type of room on CFU/m^3 air in both hospitals.
A: Bacteria, **B:** Fungi.

Table 2. Enumeration of fungi (CFU/m^3 air) according to the kind of hospital, the type of room and the time of sampling.

		Fungal CFU/m^3 air		
		Morning (n = 3) 10 - 12 am	**Afternoon (n = 3) 2 - 4 pm**	**Evening (n = 3) 7 - 9 pm**
Governmental Hospital	ICU	36	47	23
	OR	28	54	33
	NW	37	45	30
	ME	41	73	21
	PR	43	47	38
Private Hospital	ICU	21	33	21
	OR	5	3	2
	NW	27	19	28
	ME	22	29	23
	PR	35	44	9

ICU: Intensive Care Unit, **OR:** Operating Room, **NW:** Neonatal Ward, **ME:** Main Entrance, **PR:** Patient Room. LSD *p*-values from comparison of governmental and private hospitals according to type of room (ICU p =0.0907, OR p<0.000, NW p<0.0446, ME p=0.0015 and PR p=0.0291).

for the intensive care unit (ICU) (Figure 1B). In the governmental hospital, there were no significant differences between the different units. On the other hand, the operating room had the lowest rates in the private hospital.

Enumeration of microbial colonies from swab samples

Table 3 depicts the counts of bacterial swabs taken from the surfaces of the operating rooms and neonatal wards

Table 3. Enumeration of bacterial colonies from each location in the kind of hospital.

			Bacterial CFU		
			Morning (n = 3) 10 - 12 am	**Afternoon (n = 3) 2 - 4 pm**	**Evening (n = 3) 7 - 9 pm**
Governmental Hospital	Surface	OR	43	48	45
		NW	71	53	71
	Ventilation	ICU	175	229	154
		PR	205	253	188
Private Hospital	Surface	OR	25	41	21
		NW	38	32	14
	Ventilation	ICU	87	128	103
		PR	109	144	96

ICU: Intensive Care Unit, **OR:** Operating Room, **NW:** Neonatal Ward, **ME:** Main Entrance, **PR:** Patient Room. LSD *p*-values from comparison of governmental and private hospitals according to surface swabs $p< 0.000$ and ventilation swabs $p<0.0000$.

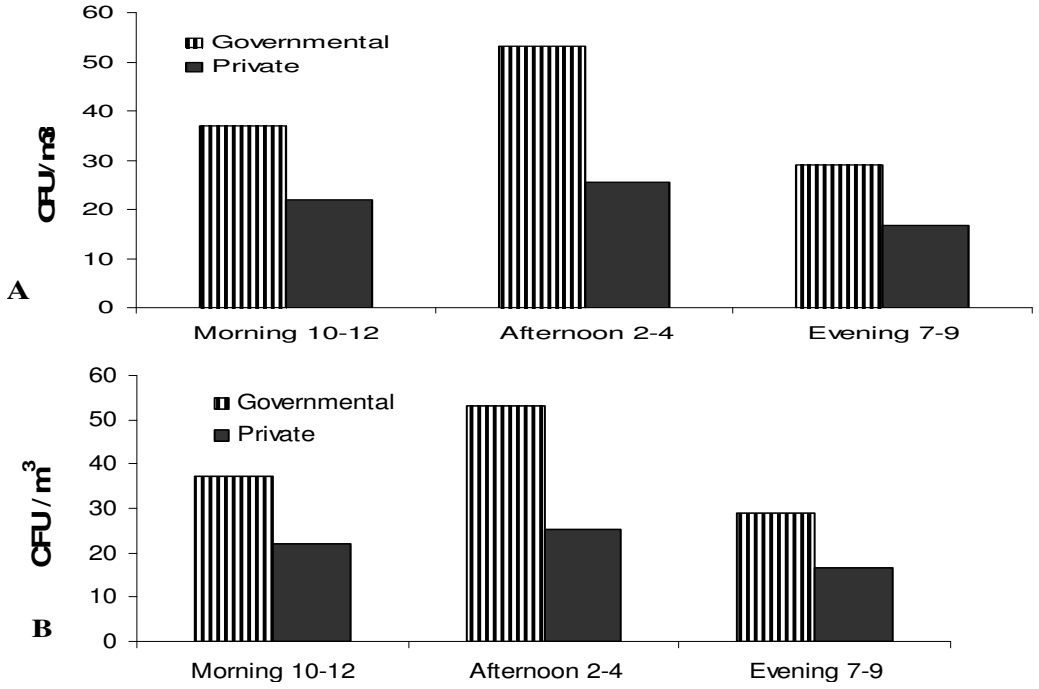

Figure 2. The effect of the kind of hospital and the time of sampling on CFU/m^3 in indoor air in both hospitals. **A:** Bacteria, **B:** Fungi
LSD *p*-values from comparison of governmental and private hospitals bacterial count according to time of morning 10 - 12 p = 0.0102, afternoon 2 - 4 $p<0.000$, evening 7 - 9 $p = 0.0098$; LSD *p*-values from comparison of governmental and private hospitals fungal count according to time of morning 10 - 12 $p = 0.0012$, afternoon 2 - 4 $p<0.000$, evening 7 - 9 $p = 0.0076$.

from the governmental and private hospitals. In the governmental hospital, the counts ranged from 43 to 71 CFU. In the private hospital, the range was between 14 to 41 CFU. Table 3 also shows the bacterial counts from the ventilation grills of intensive care units and patient rooms. The counts ranged from 154 to 253 CFU for the governmental hospital, and for the private hospital the counts ranged from 87 to 144 CFU. The fungi could not be enumerated in all locations due to the large numbers of colonies and their overlapping on the surfaces of the SDA agar plates.

The results in Figure 2 show that the governmental hospital had higher bacterial CFU/m3 air (Figure 2A) and fungal CFU/m3 air (Figure 2B) at all times and was more sensitive to the effect of sampling time compared to the private hospital. Figure 3A shows that bacterial CFU/m3 air in the main entrance and the patient rooms were more sensitive to the change in the sampling time, while the other units were not sensitive. As indicated in Figure 3B, fungal CFU/m3 air in three locations (ICU, main entrance

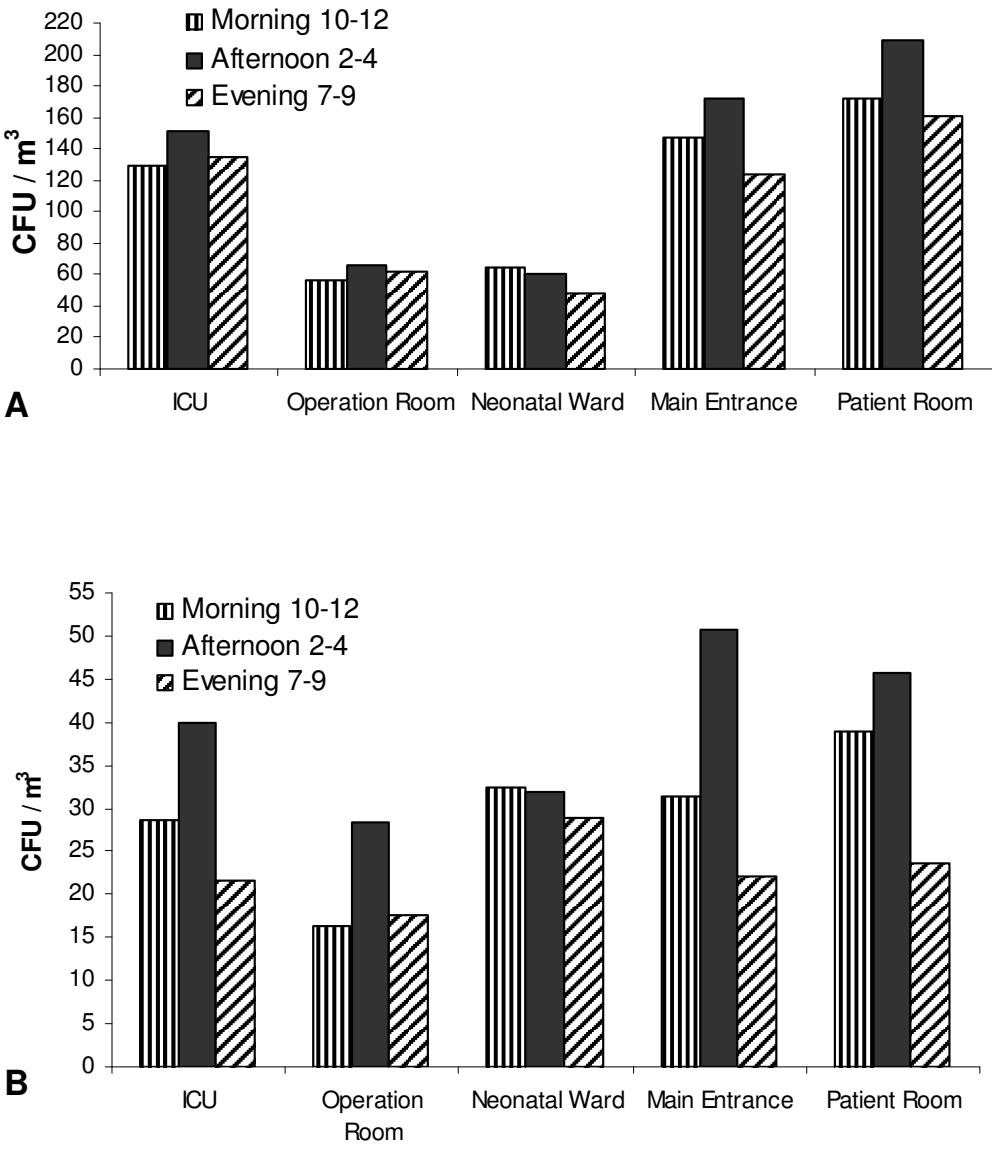

Figure 3. The effect of type of room and time of sampling on CFU/m^3 air in both hospitals.
A: Bacteria, **B:** Fungi
LSD *p*-values from comparison bacterial count, ICU morning and ICU afternoon p=0.2935, ICU morning and ICU evening p=0.7951, OR morning and OR afternoon p=0.6689, OR morning and OR evening p=0.8306, NW morning and NW afternoon p=0.8665, NW morning and NW evening p=0.4548, ME morning and ME afternoon p=0.2476, ME morning and ME evening p=0.2935, PR morning and PR afternoon p=0.0929, PR morning and PE evening p=0.6144.
LSD p-values from comparison fungal count, ICU morning and ICU afternoon p=0.1982, ICU morning and ICU evening p=0.4252, OR morning and OR afternoon p=0.1734, OR morning and OR evening p=0.879, NW morning and NW afternoon p=0.9696, NW morning and NW evening p=0.7037, ME morning and ME afternoon p=0.0298, ME morning and ME evening p=0.2884, PR morning and PR afternoon p=0.4475, PR morning and PE evening p=0.0831.

and patient room) were more sensitive to the change in sampling time, while the rest of locations were not sensitive.

The types of microorganisms isolated from the air of the five different locations are shown in Tables 4 and 5.

The largest quantities of isolated bacteria in the governmental hospital was *S. aureus* (122 CFU/m3), followed by *Micrococcus luteus* (100 CFU/m3) and coagulase negative *Staphylococci* (98 CFU/m3). Among fungi, Aspergillus spp. was the highest (60 CFU/m3) followed by Penicillium spp. (36 CFU/m3). Regarding the private hospital, the most abundant bacteria was coagulase-negative *Staphylococci* (90 CFU/m3), followed by *S. aureus* (88 CFU/m3) and *M. luteus* (56 CFU/m3), while

Table 4. Airborne microorganisms isolated from five locations in governmental hospital

Types of organisms	CFU / m^3 air (%)					
Bacteria	**ICU**	**OR**	**NW**	**ME**	**PR**	**Total**
S. aureus	26(21.3%)	14(11.5%)	22(18.0%)	0(0.0%)	60(49.2%)	122(100%)
E. faecalis	18 (47.4%)	2(5.3%)	12(31.6%)	2(5.3%)	4(10.5%)	38(100%)
M. luteus	22 (22.0%)	20(20.0%)	14(14.0%)	12(12.0%)	32(32.0%)	100(100%)
B. subtilis	24 (41.4%)	6(10.3 %)	6(10.3%)	8(13.8%)	14(24.1%)	58(100%)
B. cereus	14 (41.2%)	10(29.4%)	8(23.5%)	2(5.9%)	0(0.0%)	34(100%)
Coagulase-negative Staphylococci	14 (14.3%)	14(14.3%)	8(8.2%)	32(32.7%)	30(30.6%)	98(100%)
Unidentified Gram-positive rods	10 (25.0%)	6(15.0%)	0(0.0%)	8 (20.0 %)	16(40.0%)	40(100%)
Pseudomonas aeruginosa	6 (23.1 %)	0(0.0%)	0(0.0%)	20(76.9%)	0(0.0%)	26(100%)
Klebsiella spp.	10 (19.2%)	6(11.5 %)	2(3.8%)	24(46.2%)	10(19.2%)	52(100%)
Escherichia coli	20 (28.6%)	12(17.1%)	6(8.6%)	14(20.0%)	18(25.7%)	70(100%)
Enterobacter aerogenes	2 (11.1 %)	6(33.3%)	0(0.0%)	10(55.6%)	0(0.0%)	18(100%)
Unidentified Gram-negative coccus	0 (0.0 %)	0(0.0%)	2(25.0%)	4(50.0%)	2(25.0%)	8(100%)
Unidentified Gram-negative rods	14 (15.6%)	4(4.4%)	6(6.7%)	30(33.3%)	36(40.0%)	90(100%)
Fungi						
Aspergillus spp.	22(36.7%)	6(10.0%)	8 (13.3 %)	10(16.7%)	14(23.3%)	60(100%)
Penicillium spp.	30(83.3%)	2(5.6%)	0 (0.0 %)	4(11.1%)	0(0.0%)	36(100%)
Rhizopus spp.	2(12.5%)	0(0.0%)	4 (25.0 %)	0(0.0%)	10(62.5%)	16(100%)
Alternaria spp.	6(100.0%)	0(0.0%)	0 (0.0 %)	0(0.0%)	0(0.0%)	6(100%)

ICU: Intensive Care Unit, **OR:** Operating Room, **NW:** Neonatal ward, **ME:** Main Entrance, **PR:** Patient Room.

Table 5. Airborne microorganisms isolated from five locations in private hospital

Types of organisms	CFU / m^3 air (%)					
Bacteria	**ICU**	**OR**	**NW**	**ME**	**PR**	**Total**
Staphylococcus aureus	20(22.7%)	10(11.4%)	12(13.6%)	4(4.5%)	42(47.7%)	88(100%)
Enterococcus faecalis	8 (66.7%)	0(0.0%)	0(0.0%)	0(0.0%)	4(33.3%)	12(100%)
Micrococcus luteus	16(28.6%)	4(7.1%)	4(7.1%)	14(25.0%)	18(32.1%)	56(100%)
Bacillus subtilis	18(45.0%)	6(15.0%)	2(5.0%)	8(20.0%)	6(15.0%)	40(100%)
Bacillus cereus	10(35.7%)	0(0.0%)	10(35.7%)	4(14.3%)	4(14.3%)	28(100%)
Coagulase-negative Staphylococci	30(33.3%)	8(8.9%)	0(0.0%)	18(20.0%)	34(37.8%)	90(100%)
Unidentified Gram-positive rods	8(36.4%)	0(0.0 %)	0(0.0%)	8(36.4%)	6(27.3%)	22(100%)
Pseudomonas aeruginosa	4(8.0%)	0(0.0%)	0(0.0%)	30(60.0%)	16(32.0%)	50(100%)
Klebsiella spp.	10(35.7%)	4(14.3%)	6(21.4%)	2(7.1%)	6(21.4%)	28(100%)
Escherichia coli	12(31.6%)	4(10.5%)	2(5.3%)	6(15.8%)	14(36.8%)	38(100%)
Enterobacter spp.	0(0.0%)	2(33.3%)	0(0.0%)	0(0.0%)	4(66.7%)	6(100%)
Unidentified Gram-negative coccus	2(25.0%)	0(0.0%)	0(0.0%)	4 (50.0 %)	2(25.0%)	8(100%)
Unidentified Gram-negative rods	28(48.3%)	0(0.0%)	4(6.9%)	16(27.6%)	10 (17.2%)	58(100%)
Fungi						
Aspergillus spp.	8(14.8%)	12(22.2%)	12(22.2%)	8(14.8%)	14(25.9%)	54(100%)
Penicillium spp.	4(10.0%)	16(40.0%)	20(50.0%)	0(0.0%)	0(0.0%)	40(100%)
Rhizopus spp.	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(100%)
Alternaria spp.	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(100%)

ICU: Intensive Care Unit, **OR:** Operating Room, **NW:** Neonatal ward, **ME:** Main Entrance, **PR:** Patient Room.

for fungi *Aspergillus* spp. (54 CFU/m3) was the most abundant.

DISCUSSION

The microbiological quality of indoor air in hospitals is as much of an issue as in any other type of building, with increased emphasis because of the potential severity of the consequences of NI. Many patients are actually at increased risk of infection while in the hospital. The problems of NI are generally largest in older hospitals which may have large wards and poor or no mechanical ventilation, and the si-

tuation is even more difficult in developing countries (Obbard and Fang, 2003; Pastuszka et al., 2005).

In this study, the three investigated factors, namely the kind of hospital, the type of room and the time of sampling, individually or combined, were found to influence the microbial rate in indoor air of hospitals, which may reflect the rate of NI. The results from this study showed that the governmental hospital had a higher degree of contamination with airborne bacteria and fungi in indoor air rather than the private hospital. These high rates in the governmental hospital might be attributed to the age of the building (governmental hospital was built in 1960 while the private hospital was built in 1986), poor and deficient hygienic conditions, low degree of cleanness and minimal disinfection procedures against airborne bacteria and fungi might raise the airborne bio-contaminants. Another factor which might be involved in the latter finding is the number of beds in each hospital; the governmental hospital houses 294 beds, and the private hospital has only 80 beds, this high bed number in governmental hospital means a high number of patients, personnel, and visitors occupying the hospital building, and consequently high number in each ward of the hospital (high occupant density). And the multiple patients per room (more than one patient in each room) might raise the number of people in rooms and in the corridors. These results indicate that the kind of hospital has a significant effect that influences the rate of airborne bacteria and fungi in indoor air.

Each hospital consist of different units with different levels of healthcare services, among these units, there must be a number of highly clean or disinfected units which have to deal with severely ill patients or critical cases such as intensive care units, the operation rooms or neonatal wards. Considering the type of room (location of sampling) as a factor affecting the indoor rate of airborne microorganisms, there was a significant effect of different levels of the degree of cleanness and disinfecttion strategies, which might lead to increased bacterial rates in the patient room (Figure 1A) since this location is occupied with high number of people all of times; patients, visitors and personnel and lead to increase in-door rate of airborne microorganisms. The high number of visitors that commonly enter the patient rooms, and the amount of materials brought from out side by the visitors, such as food, fruits, and flowers, were more common in patients rooms. These are recognized source of hospital contamination (Jaffal et al., 1997). The results from this study seem to support the statement made by most of the workers that patient room had the highest total count of microorganisms. Furthermore, old and poor ventilation systems might serve as another potential source of airborne micro-organisms in intensive care units as well as patient rooms, these microorganisms might be introduced into the indoor air of hospital units. It was clear that the microbial rates of bacterial swabs from the ventilation grills were higher in the governmental hospital compared with that in the private one, in addition to the high fungal rates which were uncountable in all swabs. This indicates the significant effect of ventilation grills as a source of airborne bacteria and fungi (Chuaybamroong et al., 2008). O'Connell and Humphreys (2000) have brought the attention toward air-conditioning systems as potential microbial sources. Furthermore, extensive data showed that intensive care units (ICU's) are high-risk areas for infections caused by antibiotic-resistant bacteria that may spread to other clinical areas of the hospital (Hsueh et al., 1998; Akinci et al., 2005; O'Connell and Humphreys, 2000). Kumari et al. (1998) have also reported the role of ventilation grills as a potential source of methicillin-resistant *S. aureus*.

In the main entrance, which is the passageway between the hospital and its environment, the large numbers of patients, visitors and personnel raise the microbial rates especially at the afternoon because of the maximum activity of people there. The exchange between indoor and outdoor air raise the microbial rate brought from outside the hospital into the main entrance, and this coincides with many studies which have reported the role of outdoor microbial concentrations through opened windows and doors in raising the microbial rates and homogenization of indoor air of buildings (Jaffal et al., 1997; Hyvärinen et al., 2001; Rainer et al., 2000).

The number of microorganisms in the operation room and neonatal ward was extremely low. This was anticipated due to the high sanitary standards in this area, compared to other hospital areas. It is worth noting that microbial rates in the operation room were dependent on the hospital. The location of the operation room is very important in order to reduce the microbial exchange with the other units through the air. In the private hospital which had the lowest airborne microbial counts, operation room had a good ventilation system and was located in the basement floor away from the other units, in contrast with governmental operation room which was located near the other units of the hospital. Also neonatal ward is not occupied with large numbers of personnel. Intensive disinfection procedures are performed along the day to reduce the microbial rates as much as possible, but the efficiency of these procedures is dependent upon the kind of hospital as shown in Figures 1A and 1B. Furthermore, the bacterial swabs from surfaces in operation room and neonatal ward indicated that the resident microorganisms have a significant role in raising the bacterial rates in governmental hospital. Room sett-ings and surfaces are potential sources of microorganisms, which are always exchanged with the indoor air, higher surface microorganisms coincide with higher microbial rates in indoor air and vice a versa. Li and Hou (2003) have reported that in the hospital operating rooms in Taipei, Taiwan, the concentration of airborne bacteria also varied from 10 to 102 CFU/m3, but in the bone mar-row transplantation rooms the concentration was much lower, changing to 0 to 2 CFU/m3. Similar data have been published by other workers (Augustowska and Dutkiewicz, 2006 and Pastuszka et al., 2005; Krogulski, 2008, and

2006).

Regarding intensive care unit, this unit has to deal with critical cases and there must be sufficient strategies to reduce the microbial rates as much as possible. The microbial rates in this study showed high rates in both hospitals (with higher rates in governmental hospital as mentioned before). This might be correlated to the fact that both hospitals allow visitors to enter the ICU without any precautions.

Moreover, the governmental hospitals in Jordan usually have specific times for visiting patients (2:00 - 4:00 pm). In these times, the hospitals are crowded with the visitors in addition to the hospital employees and patients. On the other hand, the private hospitals donot have limitations about the time of visits. The time of sampling found to be more effective on the rate of indoor air microorganisms in the governmental hospital rather than the private hospital. These findings agree with the findings of other studies of earlier authors who examined microflora of air in hospitals (Augustowska and Dutkiewicz, 2006; Krogulski, 2008; McCarthy et al., 2000; Pastuszka et al., 2005) who reported on the role of hospital building and employee number in raising the airborne bacterial counts. Moreover, Li and Hou (2003) have specified that bacterial levels were found to be higher and more sensitive to the activities of personnel than fungal concentrations. In the present study, the bacterial rates were more sensitive to the number of people in each ward than fungal rates and it also agrees with the results obtained by Talon (1999), Emori and Gaynes (1993), Lemmen et al. (2004), Krogulski (2008) and Sudharsanam et al. (2008). The results obtained by these workers showed that there is mounting evidence that the environment of high number of patients colonized with bacteria serves as a potential reservoir for dispersal and hence, possible infection in the hospital environment. Obbard and Fang (2003) showed that occupant density is a key factor affecting concentrations of airborne bacteria, their results showed that occupant density was dependent upon the time and this support our findings.

In general, fungal rates were less sensitive to the type of room. This result is in line with the finding of Rainer et al. (2000) who reported that no remarkable differences could be observed in the concentration of airborne fungal propagules inside the hospital units, and in the corridors in front of it. No significant barrier to air exchange had been established between the supposedly protective area of the special care unit and the surrounding rooms. Other studies have indicated that the determination of the concentrations of airborne viable fungi is affected by activities, sources, accuracy of the sampler, growth medium used, and viability of spores (Ayliffe et al., 1999; Chuaybamroong et al., 2008; Hyvärinen et al. 2001; Krogulski, 2008; Sudharsanam et al., 2008). Moreover, Tiffany and Bader, (2000) have reported that the key to the growth and spreading of fungi in building units is a moisture supply. Fungi appear on materials and introduced into the into the indoor air in a particular succession according to their minimum moisture demands.

Considering the combination between the type of room and the time of sampling, three locations in the hospitals were influenced by the time of visit; the patient room, intensive care unit and main entrance. The presence of high numbers of external visitors in these locations at the time of visit provide a potential source of microorganisms that introduced from the outdoor environment in short period of time, while the operation room and the neonatal ward have no change in the occupant density along the day and therefore, no effect of the sampling time was observed in these units.

The airborne bacterial and fungal species which were indicated in Tables 4 and 5 were found to be suspended in indoor air of both governmental and private hospital and might be a potential source of NI in our hospitals. These species had been reported in several studies that used different isolation and identification procedures (Schaal, 1991; O'Connell and Humphreys, 2000; Vincent et al., 1995; Warris et al., 2001; VandenBergh et al., 1999; Alberti et al., 2001; Rainer et al., 2000; McCarthy et al., 2000). The number of potentially pathogenic organisms in the hospital air was high. Pathogenic organisms represented more than 35% of the total count of bacteria isolated. *S. aureus* was found to be the most common organism isolated presenting 16.2%. A similar observation was observed in the study of Jaffal, et al. (1997) and showed that *S. aureus* was more common in the pediatric and female surgical wards. The common genera of fungi frequently isolated from the hospital air, *Aspergillus*, *Alternaria* and *Penicillium*. In the present study, *Aspergillus* spp. was found to be the most common fungus isolated in the two hospitals. The study of airborne fungal spores is important to understand the dissemination, spread, and movement of the microbes, particularly the pathogenic ones in the hospital atmosphere (Augustowska and Dutkiewicz, 2006; Krogulski, 2008; Gangneux et al., 2006; Samson et al., 2002; Sudharsanam et al., 2008).

Conclusion

Nine bacterial genera and four fungal genera were isolated and identified from indoor air of hospitals in Zarqa city. The kind of hospital is a significant factor that influences the rate of indoor air microorganisms because of differences in the age of the hospital buildings, number of beds and disinfection strategies. Bacterial rates were more sensitive to the type of unit inside the hospital than fungal rates. The limitations on of the time of visits in the governmental hospitals leads to increase the number of people in hospital building in short period of time and consequently raise the airborne microbial rates at this period of time. Well-constructed ventilation systems and air-conditioning systems are needed to decrease the concentrations of microorganisms that may be introduced

into the indoor air of hospitals. The kind of hospital, the type of room and the time of sampling are three factors that affect the indoor airborne microbial rates. More studies need to be done to establish a surveillance base on the Jordanian healthcare facilities. Further studies are needed to investigate the antibiotic-resistance patterns of isolated airborne microorganisms. Implementation of more stringent, frequent and comprehensive disinfection procedures should be applied in all healthcare facilities in Jordan.

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