



Original Research Article

A Qualitative and Quantitative Study Monitoring Indoor Fungi in High Risk Patients' Units in a University Hospital, Egypt

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ABSTRACT

Keywords

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The purpose of this study was to map both qualitatively and quantitatively the fungal species present in air, on surfaces and on hands of medical staff in 3 units of Zagazig University Hospitals (ZUH) which are the intensive care unit (ICU) of cerebral stroke cases, the oncology and the haemodialysis units. Among the 3 units, ICU was the least contaminated where the mean \pm SD of air fungal load (AFL) and fungal concentration on surfaces (16.7 ± 4.8 c.f.u /m³, 941.2 ± 241.1 c.f.u /m²) were significantly lower than oncology (30.8 ± 13.8 c.f.u / m³, 2387 ± 391.2 c.f.u /m²) and hemodialysis units (38.3 ± 19.5 c.f.u /m³, 2706.7 ± 650.8 c.f.u /m²). Filamentous fungi (FF) accounted for 99% of fungi isolated from air with *Aspergillus* and *Cladosporium* predominated. *Candida albicans* was the most predominant fungus isolated from surface samples (36.9% in total). Fungal concentration on the hands of ICU staff was significantly lower ($P < 0.05$) than the staff in the other two units with *Candida* spp. predominating (80.2%) followed by *Aspergillus* spp. (19.8%). A considerable number of FF and yeasts were found in the environment of ZUH which necessitates more adherence to cleaning and disinfection measures.

Introduction

Fungi are ubiquitous in the natural environment, appearing in air, water and soil. The exposure to fungal species that contaminate buildings, building constituents and the environment is inevitable hazard especially for those with impaired host defences who suffer the most severe forms of fungal infections. However, invasive fungal infections can also occur in persons

with normal host defences and can be life threatening (Pasqualotto and Denning 2006; Salgado *et al.*, 2010).

Fungi reproduce mostly by spores which are light weight and able to travel through air. This makes them disseminate both in the indoor and outdoor environments. The indoor air environment, however, can

potentially place patients at greater risk than the outside environment as enclosed spaces can confine aerosols and allow them to build up to infectious levels (Jaffal *et al.*, 1997).

Although the relationship between airborne microbial level and the incidence of infection in hospitals is not well defined (Ortiz *et al.*, 2009), some studies have demonstrated a relationship between environmental fungal contamination in hospitals and the incidence of invasive aspergillosis (Pasqualotto and Denning 2006).

Consequently, air fungal load (AFL) and its fluctuations, as well as the predominant fungal species in the hospital environment are expected indirectly to influence the incidence of hospital-acquired fungal infections, in addition to contaminated surfaces and hands of health-care workers which are considered to be the main route of the spread of nosocomial infections (NIs) (Panagopoulou *et al.*, 2002; Garcia-Cruz *et al.*, 2012).

Several studies have shown that hospital infections can be caused by fungi, such as *Candida* spp. and various species of *Aspergillus*, *Cladosporium* and *Penicillium* with high morbidity and mortality rates in immunocompromised patients (Silveira & Husain 2008; Sipsas & Kontoyiannis 2012; Montagna *et al.*, 2012). In Zagazig University Hospitals (ZUH), information about fungal contamination in indoor environment is deficient.

The present study tried to map both qualitatively and quantitatively the fungal species present in air, on surfaces and on hands of medical staff in 3 units of ZUH which are the intensive care unit (ICU) of cerebral stroke cases, the oncology and the haemodialysis units.

Materials and Methods

Site and time of surveillance: This study was performed from the start of May 2010 to the end of April 2012, at ICU, oncology and haemodialysis units located in the 5-floor internal medicine building at ZUH. Environmental samples from air, surfaces, and hands of medical staffs were collected from each unit between 10:-- and 12:--am during the 24 months period of the study. Samples were then examined for fungal load and type in the Microbiology and Immunology Department, Faculty of Medicine, Zagazig University.

The ICU is located on the 2nd floor of the building and composed of two rooms, one 2-bed and a larger one with 26 beds. The hemodialysis unit is located in the third floor and composed of four 5-bed rooms. The oncology unit is located in the fourth floor of the building and composed of four 8- bed rooms. All the patients' rooms of the oncology and hemodialysis units depend on natural ventilation through windows while ICU has wall mounted air conditioning systems and no air exchange with outdoors by windows. All the 3 units were not equipped with HEPA filters, positive pressure or special devices for filtering the air. A reconstruction work involving the renovation of the floor and walls of the hemodialysis unit was recorded in the period between May and August 2010.

Sampling of air: Air samples (100 L each) were collected from the patients rooms once a month (24 air sample from each unit) by an impingement technique as described previously by Pepper *et al.*, (2009) with slight modifications, using a home-made device that is formed of a calibrated suction apparatus adjusted to sample 33L air per minute. The apparatus is then connected to a pre-sterilized Buchner flask containing 100

ml sterile saline. After suction for 3 minutes, saline was filtered through membrane filter with a pore diameter of 0.45 μ m (Sigma, USA). The filter was then transferred aseptically to Sabouraud dextrose agar (SDA) (Difco Laboratories, Detroit, MI, USA) supplemented with chloramphenicol (0.05 g /L) (Sigma, St Louis, MO, USA). All air samples were taken approximately at a level of 1.5 m above the floor. Events taking place such as the presence of many people, healthcare staff or operation of fans were recorded at the time of sampling.

Sampling of surfaces: Hundred and sixty one surface samples were collected from the 3 units [oncology unit (51 samples), ICU and hemodialysis unit (55 samples, each)]. Samples were collected from nursing trolleys, drug preparing tables, tables beside patients and refrigerators in addition to air conditioning systems in ICU by using a 28 cm² contact slide coated with SDA (Biotest HYCON, Cat.No.63303 Germany) for identification and counting. In addition, adhesive tapes were used to obtain direct films.

Sampling of medical staff hands: Ninety samples (30 samples from each unit) were collected from the palm surface of nurses' and doctors' dominated hands by taking a stamp from the palm using 28 cm² contact slide coated with SDA (Biotest HYCON, Cat. No.63303 Germany). All medical staff involved in this study granted their informed consent to participate.

Incubation conditions and identification of fungi: All cultures were incubated at room temperature (25-30°C) inspected daily for growth for at least 2 weeks and the number of colony forming units (c.f.u) was recorded. Then, the c.f.u in m³ of air and m² of surfaces were calculated. Colonies were then subcultured onto a potato dextrose agar for filamentous fungi (FF) or and on corn

meal agar and candida chromagar medium (HiMedia Laboratories Pvt. Ltd) for yeasts and were identified according to their macroscopic and microscopic morphologic characters.

Analysis of data: Data were analysed by SPSS (version 20). Data were presented as mean \pm SD. ANOVA and t test were used when appropriate. P value < 0.05 was considered significant.

Results and Discussion

A total of 323 samples (72 air, 161 surface and 90 palm samples) were obtained and examined both qualitatively and quantitatively.

Air samples

Fungal growth was observed in all air samples (Fig. 1). In the oncology and hemodialysis units, the highest air fungal load (AFL) was recorded in the period from May 2010 to August 2010 where AFL increased significantly (P < 0.001 each) to reach a range of 40-60 and 50-100 c.f.u /m³ compared to 10-50 and 20-50 c.f.u /m³ respectively during the remaining months of the study (Fig. 1 & Table 1). After August 2010, the AFL was low in January, February, December 2011, January and February 2012 in both units reaching 10 - 20 c.f.u /m³ and slightly higher in the remaining months reaching a range of 30-50 c.f.u /m³ in each unit (Fig. 1). On the other hand, the AFL in ICU was low ranging from 10-20 c.f.u /m³ during all months of the study.

Among the studied units, the AFL was significantly lower (P < 0.001) in the ICU compared to the other units. The highest load was recorded in the hemodialysis unit followed by that of the oncology unit (Table 1).

Six fungal genera were recovered from air samples of the three units (Table 2). FF accounted for 204 c.f.u (99%) of the total fungal air isolates (206 c.f.u) obtained from the three units along the study period. Among them, *Aspergillus* spp. dominated followed by *Cladosporium* spp. The ratio of *Aspergillus* isolates (98 c.f.u) to non-*Aspergillus* FF isolates (106 c.f.u) was approximately 1:1. *Candida albicans* (*C. albicans*) was the only yeast isolated with only 2 c.f.u (~ 1%).

Surface samples

All the 161 contact slide samples taken from surfaces in the 3 units were contaminated. The mean \pm SD of fungal concentrations on surfaces were 2387 ± 391.2 c.f.u /m² in oncology unit, 2706.7 ± 650.8 c.f.u /m² in hemodialysis unit and 941.2 ± 241.1 c.f.u /m² in ICU. The surfaces of ICU were the least contaminated among the 3 units and the difference was statistically significant ($P < 0.05$).

In oncology and hemodialysis units, the mean fungal concentration on nursing trolleys and drug preparing tables tended to be significantly lower ($P < 0.001$ each) than that of refrigerators. Similarly, in ICU, the mean fungal concentration on nursing trolleys and drug preparing tables was lower than that of the air conditioning systems (data not shown).

As a whole, five fungal genera were recovered from surface samples of the three units (Table 2). FF formed 63.1 % (570 isolates) of the total fungal isolates (903 isolates). Surface samples yielded a ratio of *Aspergillus* spp. (276 c.f.u) to non-*Aspergillus* FF (294 c.f.u) of approximately 1:1 similar to that recorded in air samples. *C. albicans* was the predominant fungus spp. isolated from surface samples in oncology

and hemodialysis units and ranked the second after *Aspergillus* spp. in ICU (Table 2).

Hands of medical staff

All the 90 hand samples were colonized with fungi. The fungi collected from nurses' and doctors' hands in the 3 units belonged to *Aspergillus* spp. and *Candida* spp. Different species of *Candida*; *C. albicans*, *C. galabrata*, *C. tropicalis* and *C. krusei* were isolated. *C. albicans* was isolated from all hand samples and it was the most predominant fungus spp. isolated in each unit (Table 2). The mean \pm SD of fungal concentration on hands was significantly lower ($P < 0.05$ each) in ICU (3.8 c.f.u \pm 1.1 /slide) than that in oncology (5 c.f.u \pm 1.5 /slide) and hemodialysis unit (5.1 c.f.u \pm 1.7 /slide).

Hospital environment is contaminated with a variety of pathogenic and non-pathogenic microorganisms that can persist on surfaces for prolonged periods. Numerous studies have demonstrated that the hands of medical staff readily acquire pathogens after coming into contact with contaminated hospital surfaces and they can subsequently transfer these organisms to the patients and inanimate surfaces that they touch (Garcia-Cruz *et al.*, 2012).

This study demonstrated that a considerable number of FF and yeasts were found in the environment of studied units in ZUH including air, surfaces and hands of medical staff. The mean AFLs in hemodialysis, oncology and ICU units were 38.3 ± 19.5 c.f.u /m³, 30.8 ± 13.8 c.f.u /m³ and 16.7 ± 4.8 c.f.u /m³ respectively. Similar studies from different countries recorded AFLs means that ranged between 0.01 and 50 c.f.u /m³ (Panagopoulou *et al.*, 2007; Ortiz *et al.*, 2009; Tormo-Molina *et al.*, 2012).

The role of demolition and building activity in raising the fungal load has been demonstrated. In these occasions, the increase in fungal load was shown to depend on the quality and strictness of protective measures (Ortiz *et al.*, 2009). In oncology and hemodialysis units, our monitoring of the monthly variation in the AFL has demonstrated a significant increase in the AFL during May to August 2010 compared to the remaining months. This period coincided with a renovation activity involving the floor of hemodialysis unit. Seasonal variation of AFL was recorded in both oncology and hemodialysis units; slightly higher AFL during spring and summer, and lower AFL during winter. This recorded seasonal variation of AFL is in agreement with findings from other countries which, however, show different seasonal patterns (Panagopoulou *et al.*, 2007; Hao *et al.*, 2011; Tormo-Molina *et al.*, 2012; Park *et al.*, 2013). On the other hand, this seasonality has not been detected in other studies (Ortiz *et al.*, 2009). In ICU, no significant monthly variation in AFL was recorded. This could be attributed to the different method of aeration in this unit. Both oncology and hemodialysis units have similar method of aeration through opened windows connected to the outside and operating fans. In addition, no working air conditioning systems were present. The ICU, that did not show similar increase in AFL and that had lower AFL during the study, has different form of ventilation which is mediated mainly by wall mounted air conditioning devices and a fanning door opening in a corridor rather than opened windows connected to the outside. Furthermore, the personal movements and visits are more restricted in the ICU than the other 2 units.

Previous studies demonstrated that a theoretical count of 10 c.f.u /m³ is

considered an accepted level of air fungal spores in non-protected environment (Latgé 1999). In our study, this count (10 c.f.u /m³) was recorded in 4 months (January, February and December 2011, and in January 2012) in oncology unit. In ICU, however, this level was recorded in 8 months (July and October 2010, January, April, July and October 2011, and February and March 2012).

Airborne spores of FF in hospital environment are expected to indirectly influence the incidence of hospital-acquired fungal infections; however, this has been difficult to prove (Alberti *et al.*, 2001). In our study, the FF isolated from air belonged to *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *Fusarium* spp. and *Trichothecium*. This finding is more or less similar to that recorded in different countries although with different percentages (Panagopoulou *et al.*, 2002; Lukaszuk *et al.*, 2007; Ekhaïse *et al.*, 2010; Hao *et al.*, 2011). Such difference may result from the variation in the environment surveyed regarding the presence of protective measures, filtration of air, age and condition of the building in addition to the difference in sampling and culturing techniques. Among the fungi isolated from air, *Aspergillus* spp. dominated with *A. flavus* forming the main bulk in oncology and hemodialysis units. *A. niger* was the most prevalent *Aspergillus* spp. in ICU. *A. fumigatus*, the most common opportunist causing diseases in immunocompromised patients, was, on the other hand, the least *Aspergillus* spp. isolated.

Of note, the black mold *Cladosporium* was the most prevalent non-*Aspergillus* FF isolated. Many studies using culture media as traps (Faure *et al.*, 2002; Panagopoulou *et al.*, 2002; Pini *et al.*, 2004; Roussel *et al.*, 2008; Tormo-Molina *et al.*, 2012) reported

Cladosporium conidia, as well as that of *Aspergillus*, to be the most frequent airborne spores in nearly all environments. On the other hand, *Penicillium* and yeasts were reported to be the predominant in other studies followed by *Cladosporium* and *Aspergillus* (Hao *et al.*, 2011; Afshari *et al.*, 2013).

The monitoring of airborne fungi can have a great importance in perspective of the prevention of hospital infections. Air sampling gives a short term estimation of environmental fungal load as spores settle after a period of time. Surface sampling is an alternative way of assessing air contamination qualitatively (Latgé 1999).

In our study, the types of fungi isolated from surface sampling almost coincided with those isolated from air. All surfaces examined in the 3 units yielded a considerable number of FF and yeasts. The five most prevalent fungi collected from surfaces in the 3 units as a whole in descending order were *C. albicans*, *Aspergillus*, *Fusarium*, *Cladosporium* and *Penicillium*, which is more or less in accordance with other studies. In Mexico, *Cladosporium* and *Microsporium canis* were reported as the main fungi isolated from surfaces followed by *Aspergillus*, *Penicillium*, *C. albicans* and *C. tropicalis* (Garcia-Cruz *et al.*, 2012). In another study carried in China, *Penicillium* and *Aspergillus* were the predominant fungi followed by *Cladosporium*, *Saccharomyces* and *Alternaria* (Hao *et al.*, 2011). This indicates that *Aspergillus*, *Cladosporium* and *Candida* constitute the major fungal genera that could be found on hospital surfaces.

This study demonstrated that the surfaces that are cleaned daily with disinfectants such as nursing trolleys and drug preparing tables were all contaminated with both FF and

yeasts where *C. albicans* and *A. niger* were isolated from almost all of them in the three units. This may indicate that damp cleaning with disinfectants is not always effective if not combined with prevention of air influx from outside and efficient air filtration. The unit with the least contaminated surfaces was the ICU. This may be explained by more adherences to protective measures in ICU.

Sampling of air condition systems was only performed in ICU. All samples yielded fungal growth and this is much higher than that detected in operating theatres in India (Kelkar *et al.*, 2005). Actually, this points to the insufficiency of cleaning and maintenance procedures of these systems.

The non-filamentous fungus (*C. albicans*), although harmless for the immunocompetent host, constitutes a potential threat to the immunocompromised patients (Walsh & Groll 1999). As a rule, candidiasis is an endogenous infection; however, exogenous infections are also possible (Kao *et al.*, 1999). Furthermore, it was demonstrated that *Candida* spp. can survive on surfaces for up to 150 days and could be involved in the development of septicaemia, urinary tract infections and surgical-site infections, among critically ill patients (Eggiman *et al.*, 2003). In this study, *C. albicans* was the major fungus isolated from surfaces. It was isolated from all refrigerators and almost all nursing trolleys and drug preparing tables in the three units in addition to other FF.

In general, all surfaces examined in this study carried pathogenic and non-pathogenic fungi. Whether the fungi recovered on the analysed surfaces were the cause of infections or not, which was not proved in this study, these surfaces form a potential source of cross-infection between the hands of health-care workers and their patients.

Table.1 Mean \pm standard deviation (SD) of air fungal load (AFL) in the three studied units (c.f.u /m³)

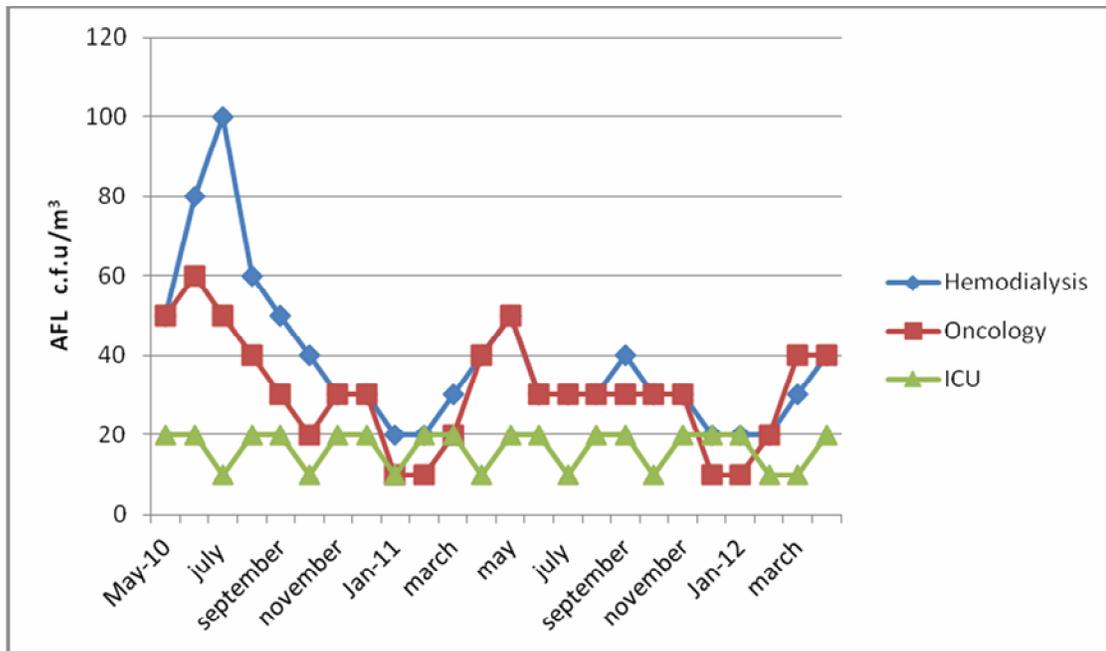
	Oncology unit	Hemodialysis unit	ICU
	Mean \pm SD (Range)	Mean \pm SD (Range)	Mean \pm SD (Range)
May 2010-August 2010	50 \pm 8.2 (40 – 60)	72.5 \pm 22.1 (50 – 100)	17.5 \pm 5 (10 – 20)
September 2010-April 2012	27 \pm 11.3 (10 – 50)	31.5 \pm 9.3 (20 – 50)	16.5 \pm 4.9 (10- 20)
May 2010-April 2012	30.8 \pm 13.8 (10 – 60)	38.3 \pm 19.5 (20 – 100)	16.7 \pm 4.8 (10 – 20)
P value	<0.001**	<0.001**	0.54

** Highly significant

Table.2 Total c.f.u count of fungal species isolated from air, surfaces and hands in the 3 units OU, oncology unit; HU, hemodialysis unit; ICU, intensive care unit

	Air samples (72)				Surface samples (161)				Hand samples (90)			
	OU No.(%)	HU No.(%)	ICU No.(%)	Total No.(%)	OU No.(%)	HU No.(%)	ICU No.(%)	Total No.(%)	OU No.(%)	HU No.(%)	ICU No.(%)	Total No.(%)
Filamentous fungi												
<i>Aspergillus</i> spp.	34(45.9)	50(54.3)	14(35)	98(47.5)	94(27.6)	114(27.3)	68(46.9)	276(30.6)	30(20.0)	37(24.0)	16(13.9)	83(19.8)
<i>A. niger</i>	5 (6.7)	————	8(20.0)	13(6.3)	47(13.8)	76(18.2)	16(11.03)	139(15.4)	15(10.0)	21(13.6)	9(7.8)	45(10.7)
<i>A. flavus</i>	17(23.0)	28(30.4)	6(15.0)	51(24.7)	47(13.8)	38(9.1)	36(24.8)	121(13.4)	15(10.0)	16(10.4)	7(6.1)	38(9.1)
<i>A. fumigatus</i>	12(16.2)	22(23.9)	————	34(16.5)	————	————	16(11.03)	16(1.8)	————	————	————	————
<i>Cladosporium</i>	21(28.4)	22(23.9)	11(27.5)	54(26.2)	41(12.0)	52(12.5)	25(17.24)	118(13.1)	————	————	————	————
<i>Fusarium</i>	11(14.9)	2(2.2)	3(7.5)	16(7.8)	41(12.0)	85(20.4)	————	126(13.9)	————	————	————	————
<i>Penicillium</i>	6(8.1)	9(9.8)	6(15.0)	21(10.2)	12(3.5)	38(9.1)	————	50(5.5)	————	————	————	————
<i>Trichothecium</i>	————	9(9.8)	6(15.0)	15(7.3)	————	————	————	————	————	————	————	————
Yeast												
<i>Candida</i> spp.	2(2.7)	————	————	2(1.0)	153(44.9)	128(30.7)	52(35.9)	333(36.9)	120(80.0)	117(76.0)	99(86.1)	336(80.2)
<i>C. albicans</i>	2(2.7)	————	————	2(1.0)	153(44.9)	128(30.7)	52(35.9)	333(36.9)	70(46.7)	61(39.6)	61(53.1)	192(45.8)
<i>C. glabrata</i>	————	————	————	————	————	————	————	————	25(16.7)	38(24.7)	22(19.1)	85(20.3)
<i>C. tropicalis</i>	————	————	————	————	————	————	————	————	10(6.6)	4(2.6)	6(5.2)	20(4.8)
<i>C. krusei</i>	————	————	————	————	————	————	————	————	15(10.0)	14(9.1)	10(8.7)	39(9.3)
Total	74(100)	92(100)	40(100)	206(100)	341(100)	417(100)	145(100)	903(100)	150(100)	154(100)	115(100)	419(100)

Fig.1 Fluctuation of air fungal load (AFL) in the 3 studied units of the hospital



All medical staff hands were colonized with fungi. The major fungal isolates belonged to *Candida* spp. including *C. albicans*, *C. galabrata*, *C. tropicalis* and *C. krusei*. This comes in accordance with previous studies in Iran and Italy (Khodavaisy *et al.*, 2011; Messano 2013). In a study in Mexico (Garcia-Cruz *et al.*, 2012), on the other hand, *Microsporum* was the predominant fungus followed by *Candida*.

To conclude, this study demonstrated the presence of excessive fungal contamination in the environment of the studied units which intensifies the importance of hand washing before and after contact with patients and various surfaces. However, health professionals' adherence to this practice has been reported to be less than 50% in ZUH as reported by our infection control team. Furthermore, more attention should be given to the routine cleaning and

disinfection measures regarding the adequacy, the length and the frequency of these measures.

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