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Fungal contamination of Azadi Teaching Hospital and Hevi Paediatric Hospital Environments, Duhok, Iraq

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Abstract
This survey study was undertaken to find out the prevalence and distribution of fungi occupying the residential surfaces in Azadi Teaching Hospital and Hevi Paediatric Hospital in Duhok province, Iraq. Three hundred samples were collected from randomly selected areas in laboratories, wards, consultation rooms, operating theatres and hospital kitchen by swabs. These samples were cultured on two selective fungal media (PDA and SDA) and also samples from air were cultured on PDA in order to determine the genus or species of the agents in the samples. The results found microorganisms that were identified included forty eight isolates in addition to non identified yeasts and sterile mycelium also detected. From 300 samples collected from period January to May 2016, molds comprised 60% and yeasts comprised 40%. All fungal isolates were identified depending on the morphological and microscopic examinations as well as biochemical tests. The environmental contaminations in Azadi Teaching and Hevi Paediatric Hospitals with fungi (*Aspergillus*) were the highest fungal isolates whereas *Candida* sp. was the highest yeast isolate.

Key words: Fungi, *Aspergillus*, *Candida*, Environmental swabs.

Introduction
Fungi are ubiquitous in the environment but they rarely cause symptoms in human beings due to the effective defence mechanisms, such as the cell-mediated response. Different fungi causing infections, some are commonly occurring infections while the others are rare[1]. Fungal contamination in healthcare facilities has been the subject of numerous studies. These have shown that high percentages of hospital infections are caused by fungi, such as *Candida* sp. and various species of *Aspergillus*, *Penicillium*, and *Cladosporium*[2]. Hospitals are considered the favourite places for the emergence of a wide variety of opportunistic and pathogenic fungi; these fungi are ubiquitous and can be acquired from host surroundings or are components of normal endogenous flora. However, any decreasing in the host organism's defensive ability due to cancer, HIV infection[3-4], medical therapy and organ transplantation may lead to uncontrolled multiplication of fungi and consequent onset of infection. Among kidney transplant recipients, 11% of infections were of fungal origin. In 47% of such cases, infection occurred during the first three months after transplantation[5]. After solid organ transplantation, 80% of fungal infections involved *Candida* and *Aspergillus* species. Among immunocompromised patients in general, the incidence of invasive Aspergillosis and other fungal diseases have increased during the last few decades which remains a serious complication and could be fatal[6].

Airborne fungal conidia are inhaled by everyone, because their concentration is high in the air[7], this may be the reason why nosocomial acquired infections and community acquired infections quite often develop in immunocompromised and in immunocompetent people[8].

The aim of this study was to determine the level of fungal contamination in Azadi Teaching Hospital and Hevi Paediatric Hospital in Duhok province.

Material and methods
Samples collection
From first January to end of May 2016, 300 samples were taken using sterile transport media swabs from various environments including operating theatres, intensive care, laboratories, outpatient departments, kitchens, and other working environments like nurses' offices, and refectories in both hospitals.

Culturing laboratory:
Swabs cultivated directly on the media culture appropriate (Sabouroad dextrose agar, Malt extract agar and Potato dextrose agar) and exposure of PDA plates to air. The dishes were incubated at 25 and 37 ^{o}C for 7-35 days. By the end of this period, the cultures examined for any growth and then sub-culture of the dishes that have shown positive results[9].

Identification of Fungi :
During incubation period, different fungal colonies were subjected to macroscopic and microscopic to observe their growth, mycelium nature and structure of hyphae. Filamentous fungal growth - as mold and yeast- that grow on SDA, MEA and PDA were sub-cultured on separate SDA culture plates. One plate was incubated at 37° C, and the remaining was incubated at 25° C[10]. Pure culture growth of each mold and yeast colony was examined under magnification for their microscopic structures and cross identified by using mycological keys manuals[11-17].

Biochemical tests used for identification of *candida* species[38].

Frequency Percentage:
The percentage frequency of species isolated was calculated by applying the following formula.

$$\text{Percentage frequency} = \frac{\text{Number of isolates of the same species}}{\text{Total number of isolates of all species}} \times 100$$

Occurrence Percentage: The percentage for the Occurrence of each species isolated was calculated according to the following equation.

$$\text{Percentage of Occurrence} = \frac{\text{Number of samples that appeared to show one type}}{\text{Total number of samples}} \times 100$$

Results and Discussion

Table-1 shows number of swabs, percentages of contamination in both Azadi Teaching and Hevi Paediatric Hospitals. As shown in the table the percentage of positive contamination in Azadi Hospital is more than the percentage of contamination in Hevi Paediatric Hospital, this could due to the high number of patients occupied in this hospital.

Table 1 - The number of swabs and percentages of contamination.

Hospitals	Azadi	Hevi	Total
Swabs	190	110	300
Positive	121	63	184
%	63.6	57.2	61.33
Negative	69	47	116
%	36.3	42.7	38.66

Table-2 shows the genera of fungi isolated from both hospitals using two media PDA and SDA by swab spreading method and exposure of plates of PDA to air for 15 minutes.

The results shown thirty two species of fungi in addition to sterile mycelium and non identified yeast have been demonstrated. Thirty species of fungi were isolated on PDA using swab method; the highest number obtained was *Aspergillus* sp. (264), *Penicillium* sp. (125), *Ulocladium* sp. (86), *Cladosporium* sp. (75), followed by *Candida* sp. (69) and Rhizopus (35).

Twenty seven species of fungi were isolated on PDA plates exposure to air, the highest number obtained was *Aspergillus* sp. (71), *Ulocladium* sp. (39), *Penicillium* sp. (30), followed by *Cladosporium* sp. (25) and *Candida* sp. (17).

Twenty two species of fungi were isolated on SDA using swab method; the highest number obtained was *Aspergillus* sp. (55), *Penicillium* sp. (39), *Candida* sp. (19), Dermatophytes (16) followed by *Ulocladium* sp. (13), and *Rhizopus* sp. (11).

The difference between the numbers of fungi isolated on both media was expected because PDA is a selective medium for luxuriant growth of yeasts and molds. Potato infusion encourages mold sporulation and pigment production even in some dermatophytes[18].

PDA enhances the production of reproductive structures (conidia) and colony colour when other media fail to do so; it is often recommended for slide culture or for inducing an isolate to exhibit a characteristic pigment[19]. Because of low pH SDA is useful for the cultivation of fungi particularly those associated with skin infections[20].

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Table (2): Isolated Fungi by different culture media.

No.	Fungal isolates	Potato Dextrose Agar swab	Potato Dextrose Agar air	Saboroud Dextrose Agar	Total
1	*Absidia* sp.	7	2	5	14
2	*Acremonium* sp.	12	0	0	12
3	*Alternaria* sp.	4	9	0	13
4	*Aspergillus* sp.	138	71	55	264
5	*Aurobasidium* sp.	0	1	0	1
6	*Candida* sp.	33	17	19	69
7	*Chaetomium* sp.	1	0	1	2
8	*Chrysosporium* sp.	1	0	0	1
9	*Cladosporium* sp.	41	25	9	75
10	*Curvularia* sp.	1	0	3	4
11	*Dermatophytes fungi*	10	7	16	33
12	*Epicoccum* sp.	1	1	0	2
13	*Emericella* sp.	2	1	0	3
14	*Exophiala* sp.	12	6	2	20
15	*Fusarium* sp.	10	6	3	19
16	*Gliaocladium* sp.	3	0	3	6
17	*Geotrichum* sp.	8	8	5	21
18	*Gymnoascus* sp.	0	1	1	2
19	*Monilia* sp.	3	1	6	10
20	*Microascus* sp.	2	3	0	5
21	*Mucor* sp.	8	8	6	22
22	*Neoscytalidium* sp.	15	3	9	27
23	*Pacilomyces* sp.	1	3	0	4
24	*Penicillium* sp.	56	30	39	125
25	*Phialophora* sp.	4	2	0	6
26	*Phoma* sp.	2	0	0	2
27	*Rhizopus* sp.	17	7	11	35
28	*Stachybotrys* sp.	12	4	7	23
29	*Sterile mycelium*(pink)	22	19	16	57
30	*Talaromyces* sp.	0	1	0	1
31	*Trichoderma* sp.	4	1	1	6
32	*Ulocladium* sp.	34	39	13	86
33	*Verticillum* sp.	1	0	0	1
34	*Yeasts black* (non identified)	0	2	27	29
Total		255	275	257	1000

Table-3 shows fungal species, frequency and occurrence percentages of the genera and species of fungi isolated during the study. From 300 swabs and air samples (28) genera and 31 species in addition to non identified yeasts and sterile mycelium has been identified.

This table shows the high frequency percentage of fungi this indicates widespread of fungi in both hospitals and may be due to appropriate environmental conditions specially temperature and humidity.

The most common fungi collected were, *Aspergillus*, *Penecillium*, *Candida*, *Cladosporium*. *Alternaria*, *Rhizopus*, *Mucor*, yeast and *Fusarium* species

Aspergillus was represented by 12 species and showed the widest diversity among all recovered genera. *A. niger, A. flavus and A. fumigatus* showed the high frequency and occurrence percentages because these species are ubiquitous and the major airborne contaminant fungi.

Some species can cause infection in humans and animals. The most common pathogenic species are *A. fumigatus* and *A. flavus*. *A. fumigatus* infections are primary pulmonary infections includes Aspergillosis, Pneumonia, Asthma and Allergy[21]. *A. flavus* cause infection of the external ear and Aspergillosis[22].

Overberger *et al*. (1995) found that 70 - 80% of the fungi in hospitals air were *Aspergillus* sp. Our study showed similar distribution of the mold and yeast species[23].

Penicillium was second in the number of species isolated and was represented by ten species. These species are usually found in indoor environment and cause infections to humans and animals.

This study has shown *Candida* fungi were isolated from indoor environment in all selected units. As a rule, candidiasis is an endogenous infection; however, exogenous infections are also possible. Numerous studies have shown evidence that *Candida* fungi are detected in the indoor air at e.g. surgical, haematological and obstetric wards; they are potential and major source of infections, especially in risk group patients[24-26].

Another yeast-like organism like *Geotrichum candidum* and *Aurobasidium* are opportunistic organism that causes pulmonary and systemic infections in human and animals.

Alternaria species are emerging as opportunistic pathogens. A case of Phaeohyphomycosis caused by *A. infectoria* in a renal transplant recipient has been reported[27].

Chrysosporium species have been isolated in this study and some were reported to cause superficial skin infection.

Microascus species are considered as potentially pathogenic to human beings and are frequently isolated from floor dusts[28-30].

Fusarium species may cause a range of opportunistic infections in humans. For example nails (onychomycosis) and in the cornea (keratomycosis or mycotic keratitis)

Zygomycetes fungi isolated in this study include *Absidia*, *Mucor* and *Rhizopus* that cause brain infections via nose[31].

Many fungi isolated from both hospitals like *A. flavus*, *A. ochraceus*, *A. parasiticus*, *A. fumigatus*, *Emericella nidulans*, *Fusarium* and *Stachybotrys* secrete secondary metabolites which harm human and animals during inhalation and are known as mycotoxins[32-33] Dermatophytes also were isolated in this study including *Microsporum*, *Epidermphyto* and *Trichophyton*; however, some people are at greater risk than others. The fungus takes advantage of skin of those patients with reduced immune capacity.

Our results in this study are in line with many studies[34-37].

Table (3) The species and genus fungal isolates and the frequency and occurrence percentages

No.	Fungi	Frequency percentage	Occurrence percentage
1	*Absida sp.*	2.0	1.0
2	*Alternaria alternate* (Fr.) Keissl		2.0
3	*Aureobasidium sp.*	3.3	1.2
4	*A.carbonarius* (Bainier) Thom	1.3	0.8
5	*A.flavus* Link	21.0	13.0
6	*A.foetidus*	1.5	
7	*A.fumigatus* Fresen	5.34	4.0
8	*A.italicum*	0.0	0.24
9	*A.japonicus* Saito	1.50	0.22
10	*A.niger* Tiegh.*nom.cons*.	63.4	50
11	*A.niveus* Blochwitz		1.0
12	*A.ochraceus* K.Wilh	4.0	1.0
13	*A.parasiticus* Speare	3.5	2.5
14	*A.vadensis* Samson ,devries,Frisvad&visser		0.8
15	*A.terreus*	0.33	0.2
16	*Penicillium aurantiogresum* Dierckx	0.4	0.2
17	*P.chrysogenum* Thom		2.0
18	*Penicillium citrinum*	7.0	5.0
19	*P.expansum*		1.0
20	*P.glabrum* (Wehmer) Westling	1.33	0.7
21	*P.greseofulvum*	1.0	
22	*P.italicum*	1.0	1.0
23	*P.naglivoience* Laxa	2.0	1.0
24	*P.oxalicum* Currie & Thom	2.66	2.0
25	*P.verrocosum* Dierckx		2.33
26	*Candida sp.*	24.0	18.0
27	*Cladosporium cladosporoides* (Fresen.) G.A. de Vries	6.33	4.0
28	*Cladosporium herborum* (Pers.) Link	3 .0	2.0
29	*Curvularia sp.*	3.0	1.0
30	*Emericella nidulans* (Eidam) Vuill	0.33	0.1
31	*Epidermphyton sp.*	1.24	0.18
32	*Exophiala sp.*	0.04	0.02
33	*Phoma glomerata* (Corda) Wollenw.&Hochapfel	0.2	0.19
34	*Fusarium oxysoprum* Schlecht	0.17	
35	*Geotrichum candidum* Link ex Fr.	2.0	1.0
36	*Gliocladium sp.*	2.0	
37	*Gymnoascus sp.*		0.46
38	*Microsporum sp.*	0.16	0.02
39	*Monilia sp.*	2.0	0.46
40	*Microascus sp.*	1.0	0.33
41	*Mucor circinelloides* Tiegh	4.0	
42	*Paecilomyces varioti* Bainier	1.6	0.33
43	*Rhizopus stolonifer* (Ehrenb.) Vuill.	1.0	
44	*Rhodotorulla sp.*	1.0	0.33
45	*Stachybotrys* sp.	3.0	
46	*Sterile mycelium*	11.0	9.0
47	*Trichoderma* sp.	1.33	0,44
48	*Trichophyton sp.*	1.33	0.48
49	*Ulocladium atrum*	0.46	0.33
50	*Yeast non identified*	1.0	0.64

Conclusion

In conclusion, the present study revealed the presence of potentially pathogenic and toxigenic fungi in both hospitals in Duhok province. Strict hygienic measures should therefore be undertaken to control the spread of these fungi in the environment and to avoid mycotic infection among the patients and staff alike. Check air conditioning systems for standing moisture and clean or replace as necessary.

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التلوث الفطري في بيئة مستشفى آزادي التعليمي ومستشفى هيفي للأطفال محافظة دهوك، العراق

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الملخص

أجريت هذه الدراسة لغرض معرفة مدى أنتشار الفطريات المستوطنة والمنتشرة في بيئة كل من مستشفى آزادي التعليمي ومستشفى هيفي للاطفال في محافظة دهوك، حيث تم جمع 300 عينة بطريقة القطن المعقم (السواب) من مناطق مختلفة وعشوائية شملت: المختبرات، الاقسام، غرف الاستشارية، غرف العمليات والمطابخ وزرعت هذه العينات على نوعين من الاوساط الفطرية الاختيارية (وسط بطاطا دكستروز اكار، وسط سابروويد دكستروز اكار) كما زرعت العينات المأخوذه من الهواء على وسط بطاطا دكستروز اكار لتحديد نوع وجنس الفطريات الموجودة في هذه العينات. وخلال هذه الدراسة تم تحديد 48 نوع فطري بالأضافة الى الخمائر والمايسيليوم. وجمعت هذه العينات (300 عينة) في فترة كانون الثاني الى ايار سنة 2016 حيث بلغت الاعفان نسبة 60% وبلغت الخمائر 40%. وتم تشخيص جميع الفطريات بالأعتماد على الفحوصات المظهرية والمجهرية بالأضافة الى اجراء الفحوصات البايوكيميائية. وأظهرت الرشاشيات (*Aspergillus*) من بين الاعفان كأعلى نسبة من الملوثات الفطرية في كل من بيئة مستقى آزادي التعليمي ومستشفى هيفي للاطفال كمان اظهرت المبيضات(*Candida sp*) وانواعها أعلى نسبة من بين الخمائر.

الكلمات المفتاحية: الفطريات، الرشاشيات، المبيضات ومسحات بيئية.