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Invasive fungal infections and patients with malignancies in upper Egypt

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Abstract

The incidence of invasive fungal infections has increased considerably in recent years. The aim of this study was to present a suitable early diagnostic procedure in immune compromised patients, using detection of fungal infection of urine samples collected from 33 patients with malignancies (from 2-89 years old), during the period from December 2012 to February 2014, from South Egypt. Fifty-three fungal species representing 14 genera were collected during this investigation from urine samples on Sabouraoud's Dextrose Chloramphenicol Agar (46 species and 12 genera) and Rose Bengal Chloramphenicol Agar media (41 species and 11 genera). *Aspergillus* (16 species), *Penicillium* (14 species), *Yeasts* (5 species) and *Cladosporium* (5 species) contributed the broadest spectra of species in all samples tested on two types of media used. Other species were represented by 13 species belonging to 10 genera. The results indicate that immune compromised patient is a suitable habitat for the growth and sporulation of different groups of fungi, both saprophytic and pathogenic. A variety of types of filamentous fungi were obtained from malignancies patients. Immunosuppressant patient's exposure for fungal infection so should be in especial care from food, drinking and air.

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Introduction

Invasive fungal infection (IFI) is among the leading causes for morbidity, mortality, and economic burden for patients with malignancies. In the past few decades, the incidence of IFI has increased dramatically. Patients with malignancies are currently at higher risk of IFI caused by fungal infection, and the incidence of IFI is highest among these patients. Invasive fungal diseases (IFD) are an important cause of morbidity and mortality in patients with malignancies diseases (Kontoyiannis *et al*., 2010; Pagano *et al*., 2010). The epidemiology of IFD in this group of severely immune compromised patients has changed substantially during the last two decades; with invasive aspergillosis (IA) being a predominant infection. The incidence of this infection can vary and is mainly based on the underlying malignancy (Pagano *et al*., 2006). Over the last years, an increasing number of infections caused by moulds has been reported: *Aspergillus* spp. seems to be the main fatal complication in patients with malignancies, but other opportunistic moulds, such as *Fusarium* spp. and Zygomycetes, have also been described (Kontoy-iannis *et al*., 2005), whereas infections caused by other filamentous fungi are still rare (Girmenia *et al*., 2005; Pagano *et al*., 2004). Invasive fungal disease (IFD) is one of the most prevalent causes of morbidity and mortality in immune compromised patients (Cornely *et al*., 2007; Vehreschild *et al*., 2010). As the group of patients at high risk of IFD is heterogeneous, with different underlying diseases, risk factors and demographic characteristics, it is not surprising that more-tailored prophylactic measures are needed. Recent research has allowed some distinctions to be made between different patient groups and various guidelines and prophylactic protocols have been developed (Pappas *et al*., 2009; Ruping *et al*., 2008).

However, there are still some patient groups, such as those with acute lymphoblastic leukaemia or patients at the aplastic phase of allogeneic stem cell transplantation, in which the use of prophylaxis needs to be better assessed.

In primary cutaneous aspergillosis (PCA), the lesion results from the direct inoculation of *Aspergillus* spores at the site of skin injury following intravenous catheter, trauma, occlusive dressings and tapes, burns or surgery. In secondary cutaneous aspergillosis, lesions are consecutive to haematogenous dissemination from a primary focus such as the lung or to contiguous spread to the skin from underlying infected structures (Del Bono *et al*., 2008; Segal, 2009). Fungal infections, also called mycoses, are important causes of morbidity and mortality in humans. Some fungal infections are endemic, and these infections are usually caused by fungi that are present in the environment and whose spores enter humans. Other fungal infections are said to be opportunistic because the causative agents cause mild or no disease in healthy individuals but may infect and cause severe disease in immune deficient persons. The human air way is continuously open to the no sterile environment where fungal spores have the potential to reach lung tissue and produce disease. In the immune compromised host, many fungi, including species of fungi typically considered nonpathogenic, have the potential to cause serious morbidity and mortality (Romani, 2008).

Invasive fungal infections (IFI) have significantly increased due to advances in medical care in the at risk immune compromised population. Fungal species are widely distributed in soil, plant debris and other organic substrates, and make up approximately 7 per cent (611,000 species) of all eukaryotic species on earth (Mora *et al.,* 2011) although only about 600 species are human pathogens (Brown *et al.,* 2012). So this study aims to present a suitable early diagnostic procedure in immune compromised patients, using detection of fungal infection of invasive fungal infections (IFI) in urine samples collected from patients with malignancies and also, purification and identification of isolated fungi using morphological features and advanced molecular techniques (PCR sequencing).

Then we can control the complications of the disease and determine the appropriate antifungal to this invasive fungal infection.

Materials and methods

Collection of clinical specimens

Thirty-three urine clinical specimens were collected from malignancy patients (age ranged from 2-89 years old), during the period from December 2012 to February 2014, from South Egypt Cancer Institute in Upper Egypt, Assiut.

Isolation media

Mid-stream urine clinical specimens were collected from the patients in sterile containers, for women periurethral area and perineum were cleaned with soapy water and thoroughly rinsed with clean water. The first few millimeters of urine should be passed into a bedpan or toilet bowl to flush out bacteria from the urethra. The midstream urine is then collected in sterile container. In case the cauterized patient, urine sample was taken from the catheter as follow; the catheter was cleaned from outside with alcohol swab and punctured with a 21 gauge needle, and urine was aspirated with syringe and collected in sterile containers then centrifuged so as to concentrate the organism allow for optimal recovery, 5 mL of each sample was centrifuged at 1500 rpm for 10 minutes, the sediment was used for culture. Clinical specimens were transferred in sterilized plastic container to the laboratory and stored at 4°C, where fungal analysis was made. The media used for the isolation of fungi were Sabouraoud's Dextrose Chloramphenicol Agar and Rose Bengal Chloramphenicol Agar media. All components of the isolation media were added prior to autoclaving at 121°C for 20 min except chloramphenicol, which was sterilized and added to the media after autoclaving. The plating technique was employed for determination of fungi of urine samples.

About 1 mL urine from each clinical specimen was scattered on the surface of each of two isolation media.

Six plates were used for each urine sample (three plates for each type of media). Plates were incubated at 30°C (for yeasts and molds isolation) and examined daily for 15 days to allow for development of pigment on colonies to facilitate complete differentiation of fungal types and the counts was calculated per 1mL urine in each clinical specimen. Repeated sub-culturing on Sabouraoud's Dextrose Chloramphenicol Agar and Rose Bengal Chloramphenicol Agar media, were essential to obtain pure cultures. Sporulation was induced by exposing the cultures to ultraviolet light. Isolates were characterized according to morphological features, cultural characteristics such as pigmentation of the mycelium and direction of growth of the hypha, whether aerial or lateral, microscopic observation of structures involved in asexual reproduction, e.g. conidia or spores, and in sexual reproduction, and the presence of fruiting bodies. Light photomicrographs were taken mostly from slide cultures. Slide cultures were performed by removing a small cylinder of the agar medium by a cork borer, and inserting it on the surface of the same agar inside a Petri dish. The top of the cylinder was inoculated with the fungus and covered with a sterilized cover slip. After few days, the fungus growing on the cover slip is gently stained with cotton blue and mounted in lactophenol. Identification was accomplished using appropriate taxonomic techniques, such as those of (AUM C, 2014; Kauffman *et al*., 2006; Moubasher, 1993; Pitt and Hocking, 2009).

The yeast cultures were identified primary in our laboratory and PCR fragments were sequenced by Sol Gene, Korea (Scherer and Stevens, 1987; White *et al*., 1990; Buitkamp, 1991). DNA sequences, reported in the current study, and were deposited in the NCBI nucleotide sequence database, Gene bank. (www.ncbi.nlm.nih.gov).

Statistical analysis.

The present study conducted an ANOVA (analysis of variance) which was performed on all the treatments and done using the SPSS (version 10.0) package to determine whether or not, a significance difference.

Results

Fifty-three fungal species representing 14 genera were collected during this investigation from urine samples on Sabouraoud's Dextrose Chloramphenicol Agar (46 species and 12 genera) and Rose Bengal Chloramphenicol Agar media (41 species and 11 genera) (Table 1, 2).

Invasive fungal infections on Sabouraoud's Dextrose Chloramphenicol Agar medium.

Fourty six species invasive fungi representing 12 genera were isolated from 33 urine sample on Sabouraoud's Dextrose Agar at 28±2°C (for yeasts and molds isolation) and examined daily for 15 days. (Table 1).

Aspergillus was the most common genus and was recovered in high frequency occurrence 69.7% of the sample constituting 45.5% of total fungi. It was represented by 16 species. *A. niger* and *A. flavus* var. *columnaris* were isolated in moderate frequency of occurrence. They were recovered in 42.4 and 33.3% of the samples, matching 22.9 and 36.8% of total *Aspergillus* and 10.5 and 17.0% of total fungi, respectively. *A. flavus* var. *flavus* and *A. terreus* var. *terreus* were isolated in low frequency. They emerged in 18.2% of samples matching 12.4 and 4.8% of total *Aspergillus* and 5.7 and 2.2% of total fungi, respectively. *A. amstelodami, A. chevalieri, A. clavatus, A. candidus, A. flavipes, A. fumigatus, A. giganteus, A. sydowii, A. terreus* var. *africanus,* and *A. terreus* var. *aureus* were isolated in rare frequency. They emerged in 3.0, 3.0, 3.0, 6.1, 3.0, 6.1, 6.1, 3.0, 9.1 and 12.1% of the samples matching 1.0, 0.1, 0.3, 1.2, 8.9, 2.9, 3.8, 0.6, 1.9, and 2.2% of total *Aspergillus* and 0.4, 0.2, 2.0, 0.6, 4.1, 1.3, 1.8, 0.3, 0.9 and 1.0% of total fungi, respectively where patient suffered from Non-Hodgkin's lymphoma and Hodgkin's lymphoma (Table 1).

Cladosporium occupied the second place in the number of cases of isolation and was recovered in high frequency of occurrence 60.6% of samples constituting 15.1% of total fungi. It was represented by 5 species and these were *C. cladosporioides, C. herbarum, C. oxysporum, C. sphaerospermum* and *C. tenuissmum* isolated in high or rare frequency of occurrence. They were recovered in 48.5, 6.1, 6.1, 9.1 and 3.0 5 of the samples, and 11.3, 0.6, 0.9, 1.5 and 0.9% of total fungi, respectively, where patient suffered from Hodgkin's lymphoma, Acute lymphoblastic leukemia and Cancer Bladder.

Penicillium occupied the third place in the number of cases of isolation and was recovered from 45.5 5 of the samples constituting 8.2% of total fungi. It was represented by 12 species of which *P. chrysogenum* was isolated in low frequency of occurrence, and recovered in 24.25 of the samples, matching 2.3% of total fungi. *P. aurantiogriseum, P. citrinum, P. crustosum, P. duclauxii, P. expansum, P. fellutanum, P. griseofulvum, P. islandicum, P. oxalicum, P. roqueforti* and *P. sclerotiorum* were isolated in rare frequency. They were recovered in 6.1, 9.1, 3.0, 3.0, 3.0, 3.0, 3.0, 3.0, 3.0, 3.0 and 3.0% of the samples, matching in 0.4, 1.2, 0.2, 0.4, 0.2, 0.4, 0.2, 0.2, 1.6, 0.7 and 0.4% of total fungi, respectively, where patient suffered from Acute lymphoblastic leukemia (Table 1).

Yeast was low frequency of occurrence. It was recovered from 15.2% of the samples constituting 24.9% of total fungi. It was represented by 4 species which *Debaromyces hansenii* was isolated in low frequency of occurrence, and recovered in 15.2% of the samples, matching 5.4% of total fungi. While *Candida glabrata, Pichia anomala* and *P. guilliermondii* were isolated in rare frequency occurrence the were recovered in 12.1, 6.1, and 9.1% of the samples matching, in 7.3, 9.2 and 2.9% of total fungi, respectively where patient suffered from Breast cancer and Neuroblastoma. *Alternaria* (*A. alternata* and *A. chlamydospora*), *Fusarium* (*F. chlmydosporum, F. oxysporum* and *F. solani*), *Acrophialophora fusispora, Mucor racemosus, Rhizopus stolonifer, Scopulariopsis candida, Gibberella fujikuroi,* and *Microsphaeropsis amaranthi* were recovered in rare frequency of occurrence, respectively (Table 1).

Table 1. Fungi isolated from urine samples on Sabouraoud's Dextrose Chloramphenicol Agar.

Isolation media Genera & species	Sabouraoud's Dextrose Chloramphenicol Agar		
	TC	F%	NCI&OR
Acrophialophora fusispora (S.B. Saksena) Samson 1970	3	6.1	2R
Alternaria	8	12.1	4R
A. alternata (Fr.) Keissl. 1912	2	3.0	1R
A. chlamydospora Mouch. 1973	6	9.2	3R
Aspergillus	315	69.7	23H
A. amstelodami L. Mangin 1908	3	3.0	1R
A. candidus Link 1809	4	6.1	2R
A. chevalieri L. Mangin 1910	1	3.0	1R
A. clavatus Desm. 1834	1	3.0	1R
A. flavipes (Bainier & R. Sartory) Thom & Church 1926	28	3.0	1R
A. flavus var. columnaris Raper & Fennell 1988	116	33.3	11M
A. flavus var. flavus Link 1809	39	18.2	6L
A. fumigatus Fresen. 1863	9	6.1	2R
A. giganteus (Mattlet) Basgal 1931	12	6.1	2R
A. niger sensu auct. pro parte, pre 2007	72	42.4	14M
A. sydowii (Bainier & Sartory) Thom & Church 1926	2	3.0	1R
A. terreus var. africanus Fennell & Raper 1955	6	9.1	3R
A. terreus var. aureus Thom & Raper 1945	7	12.1	4R
A. terreus var. terreus Thom 1918	15	18.2	6L
Cladosporium	103	60.6	20H
C. cladosporioides (Fresen.) G. A. de Vries 1952	77	48.5	16H
C. herbarum (Pers.) Link 1816	4	6.1	2R
C. oxysporum Berk. & M. A. Curtis 1868	6	6.1	2R
C. sphaerospermum Penz. 1882	10	9.1	3R
C. tenuissinum Cooke 1878	6	3.0	1R
Fusarium	8	9.1	3R
F. chlamydosporum Wollenw. & Reinking. 1925	4	6.1	2R
F. oxysporum Schltdl. 1824	2	6.1	2R
F. solani (Mart.) Sacc. 1881	2	3.0	1R
Gibberella fujikuroi (Sawada) Wollenw. 1931	3	3.0	1R
Microsphaeropsis amaranthi (Heiny & Mintz 1992)	1	3.0	1R
Mucor racemosus Fresen. 1850	6	6.1	2R
Penicillium	56	45.5	15M
P. aurantiogriseum Dierckx 1901,	3	6.1	2R
P. chrysogenum Thom 1910	16	24.2	8L
P. citrinum Thom 1910	8	9.1	3R
P. crustosum Thom 1930	1	3.0	1R
P. duclauxii Delacr. 1891	3	3.0	1R
P. expansum Link 1809	1	3.0	1R
P. fellutanum Biourge 1923	3	3.0	1R
P. griseofulvum Dierckx 1901	1	3.0	1R
P. islandicum Sopp 1912	1	3.0	1R
P. oxalicum Currie & Thom 1915	11	3.0	1R
P. roqueforti Thom 1906	5	3.0	1R
P. sclerotiorum J.F.H. Beyma 1937	3	3.0	1R
Rhizopus stolonifer (Ehrenb.) Vuill. 1902	2	6.1	2R
Scopulariopsis candida Vuill. 1911	8	6.1	2R
Yeasts	170	15.2	5L
Candida glabrata (H.W. Anderson) S.A. Mey. & Yarrow 1978	50	12.1	4R
Debaromyces hansenii (Zopf) Lodder & Kreger-van Rij 1984	37	15.2	5L
Pichia anomala (E.C. Hansen) Kurtzman 1984	63	6.1	2R
Pichia guilliermondii Wick. 1966	20	9.1	3R
Total count		**683**	
Number of genera = 14		**12**	
Number of species = 53		**46**	

Invasive fungal infections on Rose Bengal Chloramphenicol Agar medium

Fourty one species invasive fungi belonging to 11 genera was isolated from 33 urine clinical specimens on Rose Bengal Chloramphenicol Agar medium (Table 2).

Aspergillus was the most common genus was recovered in 72.7% of the sample constituting 43.7% of total fungi. It was represented by 13 species of which *A. flavus* var. *columnaris* and *A. niger* were isolated in moderate frequency occurrence.

Thy were recovered in 36.4% of the clinical specimens matching 39.3 and 28.8% of total *Aspergillus* and 17.4 and 12.8% of total fungi, respectively. *A. flavus* var. *flavus* and *A. terreus var. terreus* were isolated in low frequency. They emerged in 21.2 and 18.2% of the samples matching 14.6 and 4.0% of total *Aspergillus* and 6.4 and 1.7% of total fungi, respectively (Table 2).

While *A. chevalieri, A. clavatus, A. flavipes, A. fumigatus, A. giganteus, A. nidulans, A. sydowii, A. terreus var. africanus*, and *A. terreus var. aureus* and *A. ustus* were isolated in rare frequency. They emerged in 3.0, 3.0, 3.0, 6.2, 3.0, 3.0, 9.1 and 3.0% of the clinical specimens matching 0.4, 0.4, 0.4, 1.2, 2.8, 1.2, 0.4, 1.2, 3.2, and 2.8% of total *Aspergillus* and 0.2, 0.2, 0.5, 1.2, 0.2, 0.5, 1.4, and 1.2% of total fungi, respectively, where patients suffered from Colon cancer and Acute lymphoblastic leukemia (Table 2).

Cladosporium was ranked second in the number of cases of isolated and moderate frequency of occurrence. It was recovered from 42.4 5 of the clinical specimens constituting 11.6 5 of total fungi.

I was represented by 4 species and these were *C. cladosporioides, C. herbarum, C. oxysporum*, and *C. tenuissinum* isolated in moderate or rare frequency of occurrence. They were recovered in 39.4, 6.1, 3.0 and 3.0% of the samples, and 9.2, 1.0, 0.2 and 0.4 5 of total fungi, respectively.

Also, the data in (Table 2) showed that *Penicillium* occupied the third place in the number of cases of isolation and was recovered from 36.4 5 of the clinical specimens comprising 7.8% of total fungi.

It was represented by 10 species of which *P. chrysogenum* was isolated in moderate frequency of occurrence, and recovered in 27.3% of the samples, matching 4.3% of total fungi. *P. aurantiogriseum, P. citrinum, P. expansum, P. fellutanum, P. griseofulvum, P. islandicum, P oxalicum, P. purpurogenum* and *P. verrucosum* were isolated in rare frequency.

They were recovered in 9.1, 3.0, 3.0, 6.1, 3.0, 3.0, 3.0, 3.0, and 3.0% of the clinical specimens, matching in 0.7, 0.2, 0.4, 0.5, 0.2, 0.7, 0.2, 0.2 and 0.4% of total fungi, respectively (Table 2).

Yeast was isolated in low frequency of occurrence. It was recovered from 18.2% of the samples constituting 32.8% of total fungi. It was represented by 5 species were isolated in rare frequency occurrence, *Candida glabrata, Debaromyces hansenii, Pichia anomala* and *P. guilliermondii* were recovered in 9.1, 12.1, 6.1 3.0 and 6.1% of the clinical specimens matching, in 5.9, 8.3, 11.2, 4.5 and 2.9% of total fungi, respectively, where patients suffered from Neuroblastoma., Acute lymphoblastic leukemia and Cancer bladder.

Alternaria (A. alternata and *A. chlamydospora*), *Trichothecium roseum, Acrophialophora, fusispora, Botryotrichum piluliferum*, *Microsphaeropsis amaranthi*, *Rhizopus stolonifer Scopulariopsis candida* were recovered in rare frequency of occurrence, respectively (Table 2).

Table 2. Fungi isolated from urine samples on Rose Bengal Chloramphenicol Agar.

Isolation media	Rose Bengal Chloramphenicol Agar		
Genera & species	TC	F%	NCI&OR
Acrophialophora fusispora (S.B. Saksena) Samson 1970	1	3.0	1R
Alternaria	4	6.2	2R
A. alternata (Fr.) Keissl. 1912	3	3.0	1R
A. chlamydospora Mouch. 1973	1	3.0	1R
Aspergillus	253	72.7	24H
A. chevalieri L. Mangin 1910	1	3.03	1R
A. clavatus Desm. 1834	1	3.0	1R
A. flavipes (Bainier & R. Sartory) Thom & Church 1926	1	3.0	1R
A. flavus var. columnaris Raper & Fennell 1988	101	36.4	12M
A. flavus var. flavus Link 1809	37	21.2	7L
A. fumigatus Fresen. 1863	3	3.0	1R
A. giganteus (Mattlet) Basgal 1931	7	6.2	2R
A. nidulans (Eidam) Vuill. 1927	3	3.03	1R
A. niger sensu auct. pro parte, pre 2007	74	36.4	12M
A. sydowii (Bainier & Sartory) Thom & Church 1926	1	3.0	1R
A. terreus var. africanus Fennell & Raper 1955	3	3.0	1R
A. terreus var. aureus Thom & Raper 1945	8	9.1	3R
A. terreus var. terreus Thom 1918	10	18.2	6L
A.ustus (Bainier) Thom & Church 1926	7	3.0	1R
Botryotrichum piluliferum Sacc. & Marchal 1885	2	3.0	1R
Cladosporium	67	42.4	14M
C. cladosporioides (Fresen.) G.A. de Vries 1952	53	39.4	13M
C. herbarum (Pers.) Link 1816	11	6.1	2R
C. oxysporum Berk. & M.A. Curtis 1868	1	3.0	1R
C. tenuissinum Cooke 1878	2	3.0	1R
Microsphaeropsis amaranthi (Heiny & Mintz 1992)	3	3.03	1R
Penicillium	45	36.4	12M
P. aurantiogriseum Dierckx 1901,	4	9.1	3R
P. chrysogenum Thom 1910	25	27.3	9M
P. citrinum Thom 1910	1	3.0	1R
P. expansum Link 1809	2	3.0	1R
P. fellutanum Biourge 1923	3	6.1	2R
P. griseofulvum Dierckx 1901	1	3.0	1R
P. islandicum Sopp 1912	5	3.0	1R
P. oxalicum Currie & Thom 1915	1	3.0	1R
P. purpurogenum Flerov 1906	1	3.0	1R
P. verrucosum Dierckx 1901	2	3.0	1R
Rhizopus stolonifer (Ehrenb.) Vuill. 1902	3	3.0	1R
Scopulariopsis candida Vuill. 1911	1	3.0	1R
Trichothecium roseum (Pers.) Link 1809	6	6.1	2R
Yeasts	190	18.2	6R
Candida glabrata (H.W. Anderson) S.A. Mey. & Yarrow 1978	34	9.1	3R
Debaromyces hansenii (Zopf) Lodder & Kreger-van Rij 1984	48	12.1	4R
Pichia anomala (E.C. Hansen) Kurtzman 1984	65	6.1	2R
Pichia guilliermondii Wick. 1966	26	3.0	1R
Zygowilliopsis californica (Lodder) Kudryavtsev 1960	17	6.1	2R
Total count		**579**	
Number of genera = 14		**11**	
Number of species = 53		**41**	

Note: H= high occurrence, between 16-33 cases (out of 33); M= moderate occurrence, between 9-15 cases; L= low occurrence, between 5-8 cases; and R= rare occurrence, less than 4 cases. F%= Percentage frequency of occurrence from total samples), Total count =TC, (number cases of isolation = (NCI) and occurrence remark = OR).

The most common pathogenic fungi isolated from urine samples

Fungal distribution was varied according to the type of cancer, where patients suffered from cancer bladder disease, colon cancer, ALL disease, NHL cancer, HL disease, stomach cancer, intrabdominal lymph node, Neuroblastoma, Prostatic cancer; MUO disease, tumor of right leg, ovarian cancer and Breast cancer, the most common pathogenic fungi was *Zygowilliopsis californica, A. flavus* var. *columnaris, Pichia anomala, A. flavus* var. *columnaris, Cladosporium cladosporioides*, *Cladosporium cladosporioides*, *A. niger, Pichia anomala*, *A. flavus* var. *flavus*, *A. niger*, *A. flavus* var. *columnaris, A. flavus* var. *columnaris* and (*Candida glabrata, Debaromyces hansenii) respectively,* where organism appeared in 15, 38.5, 17.5, 25, 54, 29, 63, 53, 34, 42.8, 50, 33.3 and 31% from total colonies (Table 3).

Table 3. Fungal distribution according to the type of cancer with Urine samples.

The most common fungi	Type cancer	Percent from total isolates
A. flavus. var. columnaris	Tumor of right leg	50%
A. flavus. var. columnaris	Ovarian cancer	33.3%
A. flavus. var. *columnaris*	Colon cancer	38.5%
A. flavus. var. *columnaris*	Non-Hodgkin's lymphoma (NHL)	25%
A. flavus. var. flavus	Prostatic cancer	34%
A. niger	Intrabdominal lymph node	63%
A.niger	Malignancy of undefined primary origin (MUO) disease	42.8%
Candida glabrata	Breast cancer	31%
Cladosporium cladosporioides	Hodgkin's lymphoma (HL)	54%
Cladosporium cladosporioides	Stomach cancer	29%
Pichia anomala	Acute lymphoid leukemia (ALL)	17.5%
Pichia anomala	Neuroblastoma	53%
Zygowilliopsis californica	Cancer Bladder	15%

Discussion

The results indicate that immune compromised patient is a suitable habitat for the growth and sporulation of different groups of fungi, both saprophytic and pathogenic. A variety of types of filamentous fungi and identified yeasts were obtained from urine samples of malignancies patients. It is clear that the treatment of patients with chemotherapy induced invasive fungal infection and has an effect on the numbers and diversity of fungal colonies existing from fluids of malignancies patients, this agreed with (Kawin and Anaissie, 1999), who observed that fungal infections were much more common in patients with compromised immune system.

Aspergillus (17 species), *Penicillium* (17 species), *Yeasts* (5 species) and *Cladosporium* (5 species) contributed the broadest spectra of species in all samples tested on two types of media used. Other species were represented by 19 species belonging to 14 genera.

The fungal population on Sabouraoud's Dextrose Chloramphenicol Agar medium from malignancies patients was more than that have been isolated on Rose Bengal Chloramphenicol Agar medium. These results may be due to the differences in meteorological data. Daily mean temperature, humidity, maximum wind speed, spore counts and rainfall near the university hospital (Takahashi, 1997; Fourneret-Vivier *et al*., 2006). Some species were isolated only on Sabouraoud's Dextrose Chloramphenicol Agar (2 species from *Aspergillus* (*A. amstelodami* and *A. candidus*), *Cladosporium sphaerospermum*, 3 species from *Fusarium* (*F. chlamydosporum, F. oxysporum* and *F. solani*), *Gibberella fujikuroi Mucor racemosus*, and 4 species from *Penicillium (P. crustosum, P. duclauxii, P. roqueforti* and *P. sclerotiorum*) While 2 species from *Aspergillus* (*A. nidulans* and *A. ustus), Botryotrichum piluliferum,* 2 species from *Penicillium purpurogenum* and *P. verrucosum*),

Trichothecium roseum and *Zygo*williopsis *califonica* were encountered only from urine samples on Rose Bengal Chloramphenicol Agar medium.

The results were almost in harmony with the findings reported by (Patterson *et al*., 2000; Hachem *et al*., 2004; John and Francis, 2008; Maschmeyer *et al*., 2007; Menichetti *et al*., 1998; Morgan *et al*., 2005; Pagano *et al*., 2010; Ruangritchankul *et al*., 2015; Rath and Ansorg, 1997; Pfaller *et al*., 2004; Walsh *et al*., 2004 and 2008; Bodey *et al*., 2002; EL-mahallawy *et al*., 2002; Wingard, 1995; Hachm *et al*., 2008; Cornely *et al*., 2015), they indicated that the majority of moulds isolated of malignancies patients consisted of *Aspergillus, Fusarium*, and *Yeasts*.

Our results were almost in harmony with the findings reported by (Patterson *et al*., 2000) showed that more than 350 species that belong to the genus *Aspergillus* have been described. Only a few of them are known to be pathogenic in humans such as *Aspergillus fumigatus* which is responsible for more than 90% of invasive disease. But, Hachem *et al*., (2004) , reported isolation of *A. niger, A. nidulans, A. terreus, A. clavatus,* and *A. flavus*).

The results were almost agreed with the findings of (John and Francis, 2008; Maschmeyer *et al*., 2007; Menichetti *et al*., 1998) , they found that *Aspergillus* spp were filamentous fungi that were widely distributed in nature, particularly in soil and sites with proportion seems to had changed, as previously this strain accounted for 90% of cases; however, in a recent communication, *A fumigatus* was reported in 56% of patients, followed by *A. flavus* (19%), *A. terreus* (16%), and *A. niger* (8%), cases of disease in humans by other species have been reported (*A. amstelodami A. avenaceus, A. chevalieri, A. candidus, A. clavatus, A. fischeri, A. flavipes, A. sydowii,* and *A ustus*).

In addition, they reported more than these *Aspergillus* spp such as (*A. avenaceus, A. caesiellus, A. glaucus, A. granulosus, A. oryzae, A. quadrilineatus, A. restrictus, A. versicolor* and *A. wenti*i).

Also Morgan *et al*., (2005), isolated different *Aspergillus* species from immunosuppressed host, and reported that *A. terreus* was the second most common species, isolated with a frequency of 23%. *Aspergillus* can cause a variety of clinical syndromes ranging from mild, transient asthma to serious and disseminated disease. Also the results were similar with (Pagano *et al*., 2010), noted that fungal infections in these patients are mainly caused by *Aspergillus* spp and *Yeasts* less frequently than other agents may be involved such as those responsible for *Mucor, Rhizomucor* and *Rhizopus* spp. On the other hand, (Ruangritchankul *et al*., 2015; Rath and Ansorg, 1997; Pfaller *et al*., 2004; Walsh *et al*., 2008), reported that *Candida species* such as *C. tropicalis*, *C parapsilosis* and *C. lusitaniae* had also been implicated in fungal infections in immunocomp-romised individuals. In addition to (Walsh *et al*., 2008; Bodey *et al*., 2002; EL-mahallawy *et al*., 2002), reported that cancer patients were vulnerable to fungal infection *Candida* spp continue to be the most common fungal pathogens in patients with cancer. They account for *Candida* spp 75% of fungal infections, most of which have been attributed previously to *C. albicans*. *Candida* is the most important yeast pathogen, accounting for most invasive yeast infections (Wingard, 1995; Hachm *et al*., 2008; Cornely *et al*., 2015).

Conclusion

Mould infections are mostly air borne. Contaminated water can also play a role when aerosolized (e.g. in Showers) or in cases of submersion, food, fomites and medication are less often the cause. However, health care related outbreaks do occur Immunosuppressant patients' exposure for fungal infection so should be in especial care from food, drinking and air.

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