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Detection of Aflatoxin in *Aspergillus* Species Isolated from Immunocompromised Hospitalized Patients

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ASPERGILLUS infections have grown in importance in the last few decades. However, most of the studies have focused *on Aspergillus fumigatus*, the most prevalent species in the human infections which is followed by *Aspergillus flavus* . Eventhough *Aspergillus flavus* was more common than *A. fumigatus* in some reports. *Aspergillus niger* came next to them causing invasive aspergillosis . *Aspergillus flavus* is a widely feared fungal pathogen capable of producing aflatoxin, the most potent mycotoxin . Two hundred and fifty hospitalized patients were studied for fungal infection. Out of the collected cases 109 were positive fungal infection representing 43.6% of the total cases. The age of the patients ranged from 22 to 68 years, of which 61% were male and 39% were female. Three species of the genus *Aspergillus* were collected from 18 cases representing 16.5% of the total positive. These isolates identified as *Aspergillus flavus* (11 cases) followed by *A .niger* (5 cases) and *A. fumigatus* (2 cases) representing 10.1%, 4.6% and 1.8% , respectively. Identification was carried out using the traditional method and confirmed by the molecular techniques (amplification of the internal transcribed spacer 2 (ITS2) region and direct sequencing followed by comparative GenBank analysis). All the isolates were tested for aflatoxin production using High Performance Liquid Chromatography (HPLC). Aflatoxins B1 and B2 were produced from *A. flavus* only while *A. niger* and *A. fumigatus* were non- producers. Voriconazole (200 mg/12h for 12 weeks) and Micafungin (100-150 mg/day for 12 weeks) were successfully used for treating all the cases.

Keywords: Aflatoxin, *Aspergillus flavus*, HPLC.

The genus *Aspergillus* includes over 200 species. So far around 20 species has been reported as causative agents of opportunistic infections in man (Dagenais &Keller, 2009). *A. fumigatus, A. flavus, A. parasiticus* and *A. niger* are known to cause allergic reactions and are called allergic broncho pulmonary aspergillosis (ABPA) (Shankar *et al.*, 2004). The most frequent species of *Aspergillus* causing invasive disease include *A. fumigatus*, *A. flavus, A. niger, A. terreus*, and rarely *A. nidulans*. The most common allergens include from *A. fumigatus* and *A. clavatus*. Other than *A. fumigatus*, the mold *A. flavus* also causes a broad spectrum of disease in human beings, ranging from hypersensitivity reactions to invasive

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infection and has been considered second leading cause of aspergillosis (Denning, 1998 and Morgan *et al.*, 2005). *A. flavus* is unique in being both plant and human pathogen. *A. flavus and A. parasiticus* are the major producers of mycotoxins (aflatoxins) that contaminant cereal grains such as groundnut, maize, etc., a leading to economic losses (Shankar *et al.*, 2005). A person can inhale several hundred conidia of *A. fumigatus* per day (Latge, 1999). Although the spores of *A. fumigatus* are found in a small proportion of all the airborne spores within a hospital (approximately 0.3%). Approximately 30% to 90% of the systemic infections are due to *Aspergillus* (Brakhage & Langfelder, 2002). *A. fumigatus* has gained importance because it easily causes infection in immunocompromised patients.

Human body temperature appears to provide the ideal condition for the development of invasive disease due to *A. fumigatus* and reducing the impact by other *Aspergillus* species such as *A. flavus*, and *A. niger* (Araujo & Rodrigues, 2004). The most common clinical manifestation of infection by *Aspergillus* species is invasive aspergillosis with mortality higher than 90%. *A. fumigatus, A. flavus, A. niger* and *A. terreus* are frequently isolated from airways (nose, throat, bronchi) of such patients, and colonization could lead to invasive aspergillosis (Kosalec & Pepeljnjak, 2005). Aflatoxins (AFB1, AFB2, AFG1, AFG2, and AFM1) are mycotoxins produced by *A. flavus*, *A. parasiticus* and *A. nomius* strains. The production of the major toxins are a result of particular strains of *A. flavus*, B1 is the most common and most toxic (Hedayati *et al.*, 2007). Aflatoxins also show a wide range of immunotoxic effects, they depress phagocytosis, intracellular killing and spontaneous superoxide production of macrophages (Cusumona *et al.*, 1990 and Hinton *et al.*, 2003). Aflatoxin B1 also inhibits the production of the tumor-necrosis factor (TNF), population interleukin-1 (IL1) and IL-6 by lipopolysaccharide-stimulated macrophages (Moon *et al.*, 1999). This study aimed to study aflatoxin production from aspergilli isolated from immunocompromised hospitalized patients. In humans, *A. flavus* aflatoxin production can lead to acute hepatitis, immunosuppression, hepatocellular carcinoma, and neutropenia. It is highly possible for *A. flavus* to invade arteries of the lung or brain and cause infarction. The absence of any regulation of screening for the fungus in countries that also have a high prevalence of viral hepatitis highly increases the risk of hepatocellular carcinoma (Crawford, 2005).

Micafungin is a natural product derived from other fungi as a defense mechanism for competition of nutrients. Micafungin is produced by Coleophoma empetri (Hashimoto, 2009 and Fujie & Akihiko, 2007) . Micafungin (trade name Mycamine) is an echinocandin antifungal drug. It inhibits the production of beta-1,3-glucan, an essential component of fungal cell walls . Micafungin is indicated for the treatment of candidiasis and other opportunistic mycosis (Pappas *et al.*, 2007) . Voriconazole is a triazole antifungal medication that is generally seen in patients who are immmunocompromised, and include invasive candidiasis, invasive aspergillosis, and certain emerging fungal infections .Voiconazole has become the new standard of care in treatment of invasive aspergillosis .Voriconazole is more effective than other azole drugs in blocking sterol biosynthesis, consistent with the different antifungal potencies of these compounds (Smith *et al.*, 2006) .

Materials and Methods

Study population

This study included 250 immunocompromised hospitalized patients at Ain Shams University Hospitals (Ain Shams University) during the year June 2012- May 2013. The microbiology laboratory records were reviewed daily. The corresponding medical records were reviewed and the clinical data analyzed included demographic characteristics, site of infection, host factors and the type of underlying disease at the time of diagnosis of the infection.

Sampling, culturing and strain identification

The collected sputum, urine, blood, bile, ascetic fluid, pleural fluid, pus, and throat swab samples were directly cultured on Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA). The obtained isolates were identified through examination of micro- and macro-morphologic features in accordance with standard morphological criteria (Gonzalez *et al*., 2008; Ribes *et al.*, 2000.; Madhavan *et al*., 2011; de Hoog *et al*., 2011 and Gomes *et al.*, 2011). In addition to the traditional method of identification, molecular techniques were used by comparing the ITS1-5.8S-ITS2 rDNA region sequence data of the isolated strains with reference strains data deposited in GenBank.

Extraction of DNA

Fungal isolates were grown on PDA. DNA extracted from the fungal isolates mentioned was conducted in accordance with the instructions provided by Fermentas Genomic DNA Purification Kit #K0512 (Thermo Fischer Scientific, EU). Briefly, a sufficient inoculum was suspended in 200 µl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) in a 2.2 ml Eppendorf tube, the tubes were boiled for 3 min and then placed in ice water for 10 min. Lysis solution (400 µl) was added, the tubes heated to 65°C for 30 min and then 600 µl of chloroform were added and mixed carefully. The aqueous phase containing DNA was separated by centrifugation for 10 min at 12,000 rpm at 4°C and mixed with 800 µl precipitation solution by several inversions at room temperature for 1 min each. The tubes were then centrifuged for 10 min at 12,000 rpm at 4°C. The DNA pellets were dissolved in 100 µl of 1.2 M NaCl solution by gentle vortexing. Ice cold isopropanol (500 µl) was added to the solution, the tubes were incubated for 15 min at − 20°C and then centrifuged for 10 min at 12,000 rpm at 4°C. The DNA pellet was washed with 1 ml ice cold 70% ethanol, dried and re-suspended in sterile TE buffer.

Oligonucleotides

The oligonucleotide primers used for amplification and sequencing of the ITS regions were those described by White *et al.*, (1990). This study used ITS5 (5 -GGAAGTAAA AGTCGTAACAAGG-3) and ITS4 (5 -TCCTCCGCTTATTGATATGC-3) (Bioneer Corporation, South Korea). PCR and DNA sequencing of ITS1- 5.8S- ITS2 region rDNA of fungal species.

Amplification reactions were performed in 20 µl reaction mixture containing 2.5 µl of each primer (10 pm), 2.5 µl of genomic DNA (5 µg/ml), and one PCR-Gold Master-Mix bead (Bioron-Germany; buffers, dNTP, enzyme, stabilizers, Tris-HCl, KCl, and

$MgCl_2$). Amplification was performed with a PCR Thermal Cycler (Techne Genius - UK) using the initial denaturation at 96°C for 5 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, extension at 72°C for 80 sec, and a final extension at 72°C for 10 min. The PCR reaction products were sequenced directly using a Big-Dye terminator reagent kit including Taq polymerase and the protocol recommended by the manufacturer (Model 3100 automated DNA sequencer; PerkinElmer Inc./ Applied Biosystems – Bioneer, South Korea).

Aflatoxin production

All the *Aspergillus* isolates were tested for the production of aflatoxin based on the HPLC (Frisvad & Thrane, 1993). The analytical standards (aflatoxins B1& B2) was purchased from Sigma (St. Louis, MO), and a stock solution was prepared in acetonitrile/methanol (1:1) and stored in an amber vial in a freezer (ca-18C). Malt extract (MEA) ,liquid medium, was used for obtaining culture filtrate that is used in studying aflatoxin production by fungal tested strains.

High performance thin layer chromatography (HPLC)

After 14 days of incubation, the mate and the filtrate were defatted with n-hexane extracted using chloroform. The chloroform layer was collected then evaporated and concentrated then re- dissolved in 1 ml chloroform.

Twenty microliters of the extracted samples were applied to HPLC plates (20cm x20 cm, silica gel 60 precoated plate, Merck Darmstadt, Germany) and developed with a 5:4:1 (v/v/v) mixture of toluene: ethyl acetate : formic acid for 17 cm then scanned using CAMMAG TLC scanner system at the Regional Centre for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Aflatoxin analogues were detected as a bluish spot under UV-A (365 nm) illumination and were compared with authentic standard as well as griseofulvin (Frisvad & Thrane, 1993).

Results

Identification of isolates

Out of 250 hospitalized patients included in this study, they were categorized according to their underlying diseases (Table 1). Of the collected samples, a total of 109 cases (43.6%) were positive for fungal infection while 141(56.4%) were considered negative. The highest number of patients positive for fungal infections were diabetes mellitus and chronic pulmonary disease patients (25 cases) and the least number of positive fungal infections were among liver cell failure (LCF) patients (2 cases) (Fig. 1). The recorded positive percentages among the studied patients showed high significance (*P* value <0.001).

Three species of *Aspergillus* were recovered (16.5%) (Fig. 2). *Aspergillus flavu*s was the most frequently recovered since it was isolated from 11 cases (10.1%) followed by *Aspergillus niger* 5 cases which represented (4.6%) while *Aspergillus fumigatus* was isolated from 2 cases representing (1.8%). Concerning the site of infection 9 cases of *Aspergillus* were recovered from pulmonary infection, 7 cases renal infection and 2 cases cutaneous infection (Tables 2, 3).

TABLE 1. Frequency of fungal diseases among the study population .

Underlying Diseases	Total	Positive	Negative
D M &COPD	84	25	59
Hematological malignancy	45	22	23
D. Foot	30	6	24
RF	26	15	11
Solid organ transplantation	16	14	2
Bronchogenic carcinoma	16	13	3
Lymphoma	16	7	9
Chronic-granulomatous disease	11	5	6
LCF	6	2	4
Total	**250**	**109**	**141**

D M: Diabetes mellitus**, COPD:** Chronic obstructive pulmonary disease**., RF:** Renal failure**; LCF:** Liver cell failure**; DF:** Diabetic foot.

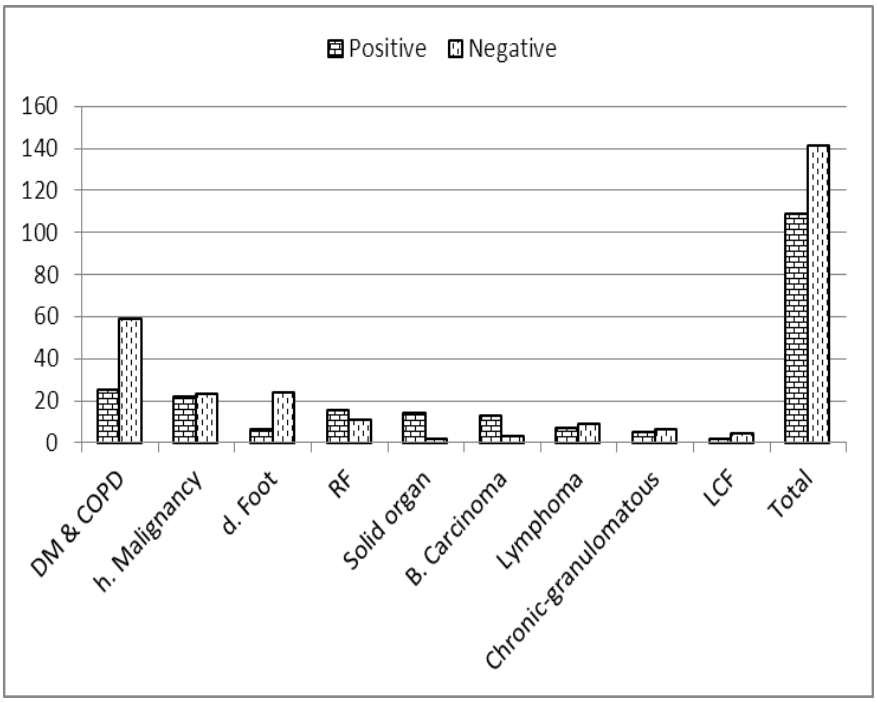

Fig. 1. Frequency of fungal diseases among the population study .

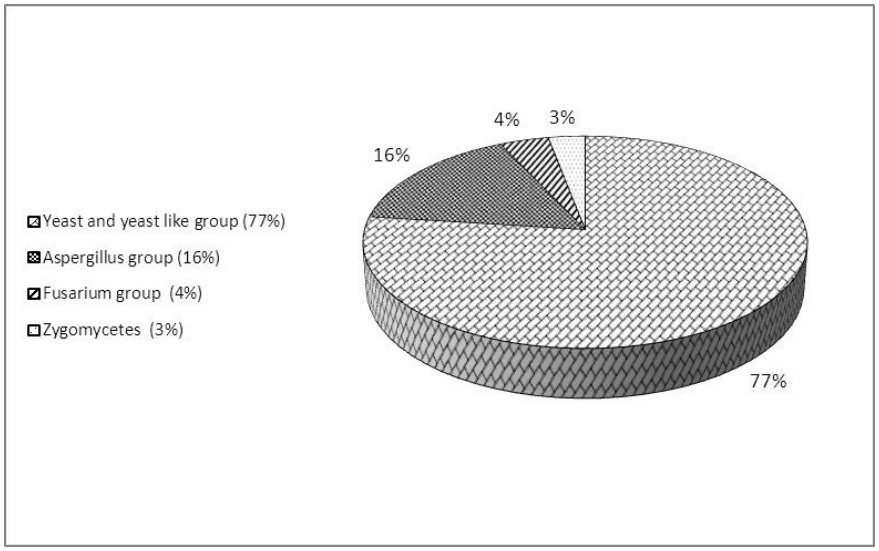

Fig. 2. Etiologic groups recovered from positive cases.

TABLE 2. Frequency of aspergillosis respective to infection sites.

No	Etiologic agent	Clinical presentation				Total	%
		Pulmonary	Renal	Disseminated	Cutaneous		
1	*Aspergillus flavus*	4	5	-	2	11	10.1
2	*Aspergillus niger*	4	1	-	-	5	4.6
3	*Aspergillus fumigatus*	1	1	-	-	2	1.8
Total		9	7	-	2	18	

TABLE 3. Frequency of aspergillosis respective to underlying condition.

Underlying Diseases	Etiologic agent			Total
	A. flavus	*A. niger*	*A.fumigatus*	
D M &COPD	2	2	-	4
Hematological malignancy	3	-	-	3
D. Foot	2	-	-	2
RF	-	-	1	1
Solid organ transplantation	1	-	-	1
Bronchogenic carcinoma	2	2	-	4
Lymphoma	-	1	1	2
Chronic-granulomatous disease	-	-	-	-
LCF	1	-	-	1
Total	**11**	**5**	**2**	**18**

Molecular identification of the recovered isolates based on the sequence of the ITS1- 5.8S-ITS2 ribosomal DNA region revealed that the *Aspergillus flavus* was highly identical (>99%) to the reference strain *Aspergillus flavus* (GenBank Accession No. JX028197) and the pair wise alignment between the recovered strain and the reference strain through blast homology search on gen bank database (NCBI) is shown in Fig. 3.

CLUSTAL W (1.83) multiple DNA sequence alignment

```
A. flavus recovered     CTCCCCCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATG
                        GCCGCCGG
A. flavus reference     CTCCCCCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGC
                        CGCCGG
                        ************************************************************************
A. flavus recovered     GGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGT
                        CTGATCTAGTG
A. flavus reference       GGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCT
                          GTCTGATCTAGTG
                          ************************************************************
A. flavus recovered     AAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCT
                        CTTGGTTCC
A. flavus reference     AAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGG
                        TTCC
                        ************************************************************************
A. flavus recovered     GGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGA
                        ATTCCGTGAA
A. flavus reference     GGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCA
                        GAATTCCGTGAA
                        ************************************************************************
A. flavus recovered     TCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATG
                        CCTGTCCGA
A. flavus reference     TCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCT
                        GTCCGA
                        ************************************************************************
A. flavus recovered     GCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTC
                        CGGGGG
A. flavus reference     GCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTC
                        CGGGGG
                        ************************************************************************
A. flavus recovered     GGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTAT
                        GGGGCTTTGT
A. flavus reference     GGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGG
                        GGCTTTGT
                        ************************************************************************
A. flavus recovered     CACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTT
                        TTTCCAGGTTG
A. flavus reference     CACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCC
                        AGGTTG
                        ************************************************************************
A. flavus recovered     ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAA
A. flavus reference     ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAA
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Fig. 3. Interspecific alignments of the 5.8S ribosomal DNA and the flanking internal transcriped spacers (ITS1 and ITS2) of *Aspergillus flavus* (GenBank Accession No.JX028197) and recovered *Aspergillus flavus* strain.

Aspergillus niger was highly identical (>99%) to reference strain *Aspergillus niger* (GenBank Accession No.KF358715) and the pair wise alignment between the recovered strain and the reference strain through blast homology search on gen bank database (NCBI) is shown in Fig. 4.

CLUSTAL W (1.83) multiple DNA sequence alignment

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A.niger recovered    AGTGCGGGTCCTTTGGGACCCAACCTC

A.niger reference    AGTGCGGGTCCTTTGGGACCCAACCTC
                      ********************************

A.niger recovered    CCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGC
                     CGCCGGGG

A.niger reference    CCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCG
                     GCCGCCGGGG
                      ************************************************************

A.niger recovered    GGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGGAGACCCCAACACGA
                     ACACTGTCTGAA
                     ************************************************************

A.niger reference    GGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCA
                     GAATTCCGTGAA

A.niger recovered    AGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATG
                     GATCTCTTG

A.niger reference    AGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATG
                     GATCTCTTG
                      ************************************************************

A.niger recovered    GTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATT
                     GCAGAATTCA
A.niger reference    GTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATT
                     GCAGAATTCA
                      ************************************************************

A.niger recovered    GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
                     GGCATGCCTG
A.niger reference    GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
                     GGCATGCCTG
                      ************************************************************

A.niger recovered    TCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCG
                     TCCCCCTCTCC

A.niger reference    TCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCG
                     TCCCCCTCTCC
                      ************************************************************

A.niger recovered    GGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGA
                     GCGTATGGGGCT
A.niger reference    GGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGA
                     GCGTATGGGGCT
                      ******************************************************

A.niger recovered    TTGTCACATGCTCTGTAGG
A.niger reference    TTGTCACATGCTCTGTA
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Fig. 4. Interspecific alignments of the 5.8S ribosomal DNA and the flanking internal transcriped spacers (ITS1 and ITS2) of *Aspergillus niger* (GenBank Accession No.KF358715) and recovered *Aspergillus niger* strain .

Aspergillus fumigatus was highly identical (>99%) to reference strain *Aspergillus fumigatus* (GenBank Accession No. KF201647) and the pair wise alignment between the recovered strain and the reference strain through blast homology search on gen bank database (NCBI) is shown in Fig. 5.

CLUSTAL W (1.83) multiple DNA sequence alignment

```
A.fumigatus recovered   CCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTTTCG

A.fumigatus reference   CCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTTTCG
                        *************************************************
A.fumigatus recovered   ACGGCCGCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGA
                        AGACCCCAACATGAA

A.fumigatus reference   ACGGCCGCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGA
                        AGACCCCAACATGAA
                        *************************************************
A.fumigatus recovered   CGCTGTTCTGAAAGTATGCAGTCTGAGTTGATTATCGTAATCAGTTAAA
                        ACTTTCAACAA
A.fumigatus reference   CGCTGTTCTGAAAGTATGCAGTCTGAGTTGATTATCGTAATCAGTTAAA
                        ACTTTCAACAA
                        *************************************************
A.fumigatus recovered   CGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGAT
                        AAGTAATGTGAAT

A.fumigatus reference   CGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGAT
                        AAGTAATGTGAAT
                        *************************************************
A.fumigatus recovered   TGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCC
                        TGGTATTCCGGG

A.fumigatus reference   TGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCC
                        TGGTATTCCGGG
                        *************************************************
A.fumigatus recovered   GGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTG
                        TGTTGGGCCCCCG
A.fumigatus reference   GGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTG
                        TGTTGGGCCCCCG
                        *************************************************
A.fumigatus recovered   TCCCCCTCTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCG
                        CGTCCGGTCCTCGAG

A.fumigatus reference   TCCCCCTCTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCG
                        CGTCCGGTCCTCGAG
                        *************************************************

A.fumigatus recovered   CGTATGGGGCTTTGTCACCTGCTCTGTAGGCCCGGCCGGCGCC
A.fumigatus reference   CGTATGGGGCTTTGTCACCTGCTCTGTAGGCCCGGCCGGCGCC
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Fig. 5. Interspecific alignments of the 5.8S ribosomal DNA and the flanking internal transcriped spacers (ITS1 and ITS2) of *Aspergillus fumigatus* (GenBank Accession No.KF201647) and recovered *Aspergillus fumigatus* strain.

Aflatoxin analysis

Among the 18 isolates of *Aspergillus* tested for aflatoxin production by HPLC method, *A. flavus* isolates were the aflatoxin producers, while *A. niger* and *A. fumigatus* were non producers (Fig. 6, 7). The result of scoring the intensity of the bands on HPLC plates revealed that all aflatoxinogenic isolates were able to yield aflatoxins B1 and B2 .

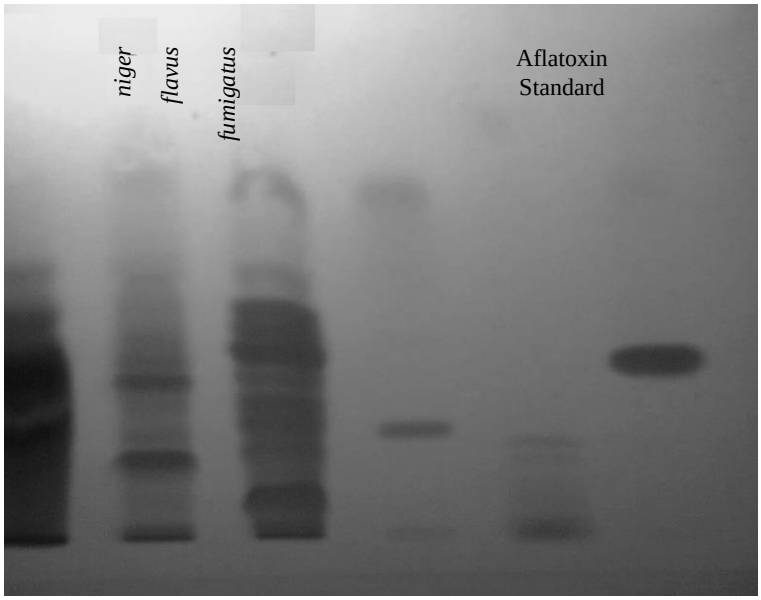

Fig. 6. Blue fluorescence aflatoxins B1and B2 produced by *Aspergillus flavus* while *A.niger* and *A. fumigatus* are negative by HPLC.

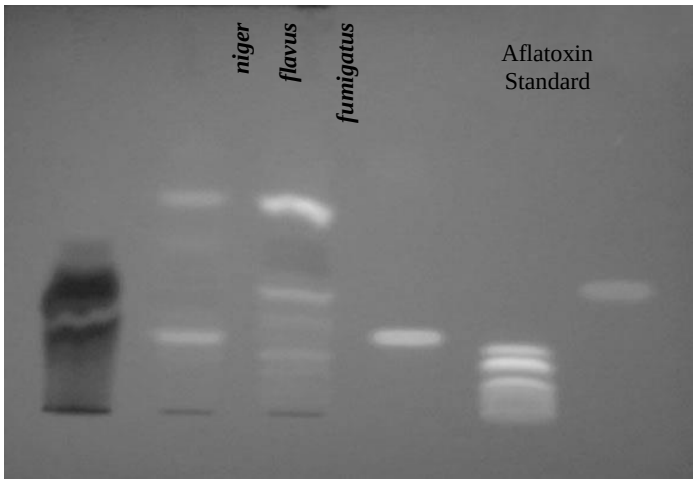

Fig. 7. HPLC analysis of aflatoxins production B1, B 2 of *Aspergillus flavus* with aflatoxin standard.

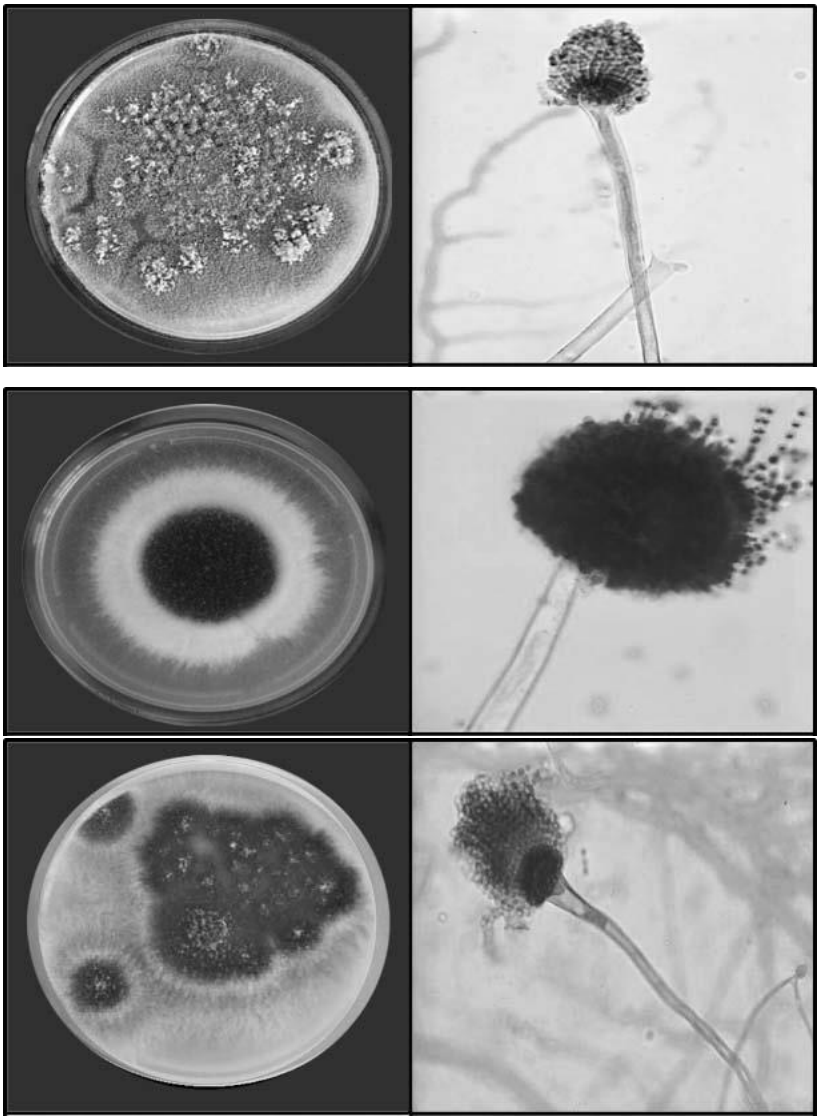

Photo (1A)

Photo (1B)

Photo (1C)

Photo 1A. Macroscopic and microscopic characteristics of *Aspergillus flavus*. Yellowish-green colony, radiate conidial heads, conidiogenous cells are uni and bi-seriate. Conidiophores are rough-walled, hyaline. Vesicles are spherical, conidia echinulate and spherical.

Photo 1B. Macroscopic and microscopic characteristics of *Aspergillus niger*. Black colony, radiate conidial heads, conidiophores are smooth-walled, hyaline or pigmented. Vesicles are sub spherical, conidiogenous cells are bi-seriate, metulae are twice as long as the phialides. Conidia are brown, ornamented with warts.

Photo 1C. Macroscopic and microscopic characteristics of *Aspergillus fumigatus*. Dark blue-green colony, columnar conidial heads, conidiophores are smooth-walled. Vesicles are sub clavate conidiogenous cells are uni-seriate. Conidia are verrucose and subspherical.

Treatment and outcome

All the diagnosed patients with aspergillosis in this study received specific therapy of antifungal as Micafungin in patients infected with yeast and yeast like fungi (100-150 mg/day for 12 weeks) and Voriconazole in patients infected with *Aspergillus* species (200 mg/12 h for 12 weeks). The median daily dose of Micafungin was 100-150/day and Voriconazole 200 mg/12 h, and the median duration of treatment was 41 days (range, 35–49 days).

Discussion

Weakening of specific immunological and non-specific host defences may predispose *Aspergillus* infections in debilitated and immunocompromised patients in hospitals (Kothary *et al*., 1984 and Bondy& Pestlea, 2000). One hundred and nine cases (43.6%) were positive fungal infection out of 250 immunocompromised patients diagnosed in the present study. Multiple risk factors in patients of this study were, D M & COPD (33.6%), chronic granulomatous disease (4.5%), LCF (2.3%), RF (10.4%), Solid organ transplantation (6.3%), Bronchogenic carcinoma (6.3%), Lymphoma (6.3%), haematological malignancies (18.1%), and diabetic foot (11.8%) which is in line with Meersseman *et al*.(2004), Vandewoude *et al*. (2006) and Prakash *et al*. (2014). Prakash *et al.* (2014) documented 17 positive cases of *Aspergillus* spp. out of 103 immunocompromised and 7 immunocompetent cases. Based on the data of the clinical history of their study, the various risk factors like pulmonary tuberculosis (9 of 33), diabetes mellitus (1 of 21), HIV infection (1 of 4), chronic smoking (4 of 42), bronchogenic carcinoma (3 of 6), bronchial asthma (3 of 15), pleural effusion (0 of 24), environmental exposure to asbestos, cement and other chemicals (2 of 22) and non significant factors (1 of 31). In this study 18 positive cases of *Aspergillus* spp among 250 immunocompromised patients as various risk factors like COPD and D. M (4 of 84), solid organ transplantation (1 of 16), bronchogenic carcinoma (4 of 16), haematological malignancies (3 of 45), lymphoma (2 of 16), chronic granulomatous disease (0 of 11), R F (1 of 26), LCF (1 of 6) and diabetic foot (2 of 30). In the present study three species of *Aspergillus* were identified (16%), *Aspergillus flavu*s was the most frequently recovered since it was isolated from 11 cases (10.4%) followed by *Aspergillus niger* from 5 cases (4.2%) and *Aspergillus fumigatus* was isolated from 2 cases (2.1%). Carpagnano *et al*. (2014) confirmed 5 cases (11.6%) of *A. niger* and 3 cases (6%) of *A.ochraceus* out of 43 lung cancer patients while the present results showed that, 2 cases (1.8%) of *A. flavus* and 2 cases (1.8%) of *A. niger* were isolated from 16 bronchogenic carcinoma patients . *Aspergillus* species were isolated from 24 cases (23.3%) out of 103 studied immunocompromised hospitalized patients,. *Aspergillus fumigatus* was the predominant species isolated from 13 cases (54.16%) followed by *Aspergillus flavus* from 7 cases (29.16%) , *Aspergillus niger* from 3 cases (12.5 %) and *Aspergillus terreus* from 1 case (4.16%). The results of this study revealed that the prevalence of pulmonary aspergillosis was 8.2% while others reported 8% (Pepy *et al.*, 1959), 8.2 (Campbell& Clayton, 1964), 11% (Henderson *et al.*, 1968) and 23.3% (Prakash *et al.*, 2014). Haq *et al. (*2007) described a case of localized renal aspergillosis in

diabetic patients, Washawasky *et al*. (1975) and Godec *et al*. (1989) reported few cases of renal aspergillosis, while 7 cases were reported in the present study. Urinary tract aspergillosis due to *A. flavus* is rare with few reported cases (Khan *et al.,* 1995; Perez-Arellano *et al*., 2001 and Kueter *et al.*, 2002) as we isolated 5 cases of *A. falvus* in this study.

Aflatoxins are secondary metabolites produced namely by members of the *Aspergillus flavus* and *A. parasiticus* (Hedayati *et al*., 2007), and they cause diseases in poultry and domestic animals (Bondy & Pestlea, 2000). However, little is known about production of aflatoxins by clinical isolates of *A. flavus* (strains isolated from immunocompromised patients). Shanker (2013) reported that *A. flavus* and *A. fumigatus* produce gliotoxin and aflatoxin *in vivo*, allow invasion in the host and they are involved in immunosuppression of the host contributing to pathogenesis. Kosalec & Pepeljnjak (2005) detected aflatoxin B1 in 7 cases (23%) and aflatoxin G1 in one case (3%) out of 30 clinical isolates of *A. flavus* collected from immunocompromised patients in a haematological unit. Also they detected aflatoxins B1 and G1 in 11 cases (37%) and one case (3%) out of 30 environmental isolates of *A. flavus*. Considering this, in the present study *A. flavus* produced aflatoxins B1 and B2 while isolates of *A. niger* and *A. fumigatus* were not producers of aflatoxins. In recent years treatment with Voriconazole controlled aspergillosis in immunosuppressed patients (Patterson *et la.,* 2005 and Agarwal & Singh, 2006). In this study the 18 cases with *Aspergillus* infection were successfuly treated with Voriconazole. Herbrecht *et al*. (2002) reported that Voriconazole proved superior to Amphotercin B with 53% complete or partial response, compared with 32% for Amphotercin B .

Conclusion

In this study *Aspergillus flavus* was the most frequent *Aspergillus* spp. causing human infections. The importance of this fungus increases in regions with a dry and hot climate. *A. flavus* isolates produce aflatoxins, the most potent hepatocarcinogenic natural toxin. In this study the *in vitro* production of toxic secondary metabolites – aflatoxins from the collected isolates of *Aspergillus* spp. isolated from immunocompromised patients was investigated. Results of aflatoxin production, including aflatoxin B1 and B2, was produced by *A. flavus* isolates only. In conclusion, the incidence of *Aspergillus flavus* was relatively high in this study and proved its ability of afaltoxin production. More investigations are needed to clear the role of aflatoxin in pathogenesis. Voriconazole proved its efficacy in treating the aspergillosis cases in this study .

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الكشف عن الأفلاتوكسين فى فطر الأسبرجلس المعزول من مرضى نقص المناعة

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ازدادت اهميه عدوى الرشاشيات في السنوات الأخيرة. ومع ذلك ، فإن معظم الدراسات ركزت على *الاسبرجلس فوميجاتوس*، والأنواع الأكثر انتشارا في هذ الجنس. وقد وجد في بعض المستشفيات في مصر أن *الاسبرجلس فلافس* أكثر شيوعا يليه *الاسبرجلس فوميجاتوس* ثم يأتي *الاسبرجلس نيجر* بعدهم فى الترتيب . ومن المعروف ان *الاسبرجلس فلافس* قادرعلى إنتاج السموم الفطرية، الأفلاتوكسين . تمت الدراسة على 250 مريضى يعانون من نقص المناعة بمستشفيات جامعه عين شمس (مصر) ، وتم دراسة العدوى الفطرية وتم تحديد إمكانية إنتاج الأفلاتوكسين من كل العزلات باستخدام جهاز HPLC وتأكيد التعريف بالطرق الجزيئية يليها تحليل بنك الجينات المقارن وقد أظهرت النتائج أن 109 (43,6٪) من الحالات كانت ايجابيه للعدوى الفطرية ، في حين كانت 141 (56,4٪) سلبي. وكان المدى العمري (22- 68) ، منها 61٪ من الذكور و 39٪ كانت للإناث. تم تعريف جنس *الاسبرجلس* وهو ما يمثل 18 حالة (16,5٪) من اجمالي الحالات الايجابيه *الاسبرجلس فلافس* 11 حالة (10,1٪) ، يليها 5 حالات (4,6٪) *الاسبرجلس نيجر* ، في حين *الاسبرجلس فوميجاتوس* معزوله عن حالتين (1,8٪). النتائج اثبتت قدره *الاسبرجلس فلافس* على انتاج نوعي B1، B2 من الافلاتوكسينات في حين أن عزلات *الاسبرجلس فوميجاتوس والاسبرجلس نيجر* المعزوله في هذه الدراسه كانت غير قادره على انتاج الأفلاتوكسينات . وقد استخدم الفريكونازول والميكافنجين بنجاح فى علاج جميع الحالات.