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In Vivo Pathogenicity of *Alternaria Chlamydospora* Isolated from Indoor Air of Liver Intensive Care Unit

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THE QUALITY of an indoor air environment is altered by the existence of microorganisms containing fungi and bacteria. This work was designed to evaluate the mycological indoor air quality of the Liver Intensive Care Unit at Suez Canal University Specialized Hospital, Egypt, along one year using conventional methods. Fourteen fungal species were isolated and identified as *Aspergillus niger, Penicillium* sp*., Alternaria* sp*., Cladosporium* sp*.,* and *Stemphyllium* sp*.* A new record isolated from air, namely *Alternaria chlamydospora*, was identified using sequencing ITS and 18S rDNA regions and its pathogenicity was studied on male and female albino rats to clarify its aggressiveness. Several parameters of blood biochemical analysis containing hemoglobin (HGB), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), white blood cells (WBC), mean corpuscular hemoglobin (MCH), red blood cell distribution width (RDW) and total count of the leukocytes (TLC) were measured. In addition, creatinine (Creat), glutamate oxaloacetate transaminases (GOT), and glutamate pyruvate transaminase (GPT) were estimated. Different organ histological sections were used for evaluation of *A. chlamydospora* pathogenicity.

The infected rat groups (two groups) injected intraperitoneally showed increase in liver and kidney enzymes in comparison to the uninfected control groups. Many severe histopathological changes (in liver, lung, pancreas, and heart) were shown in both sexes, which confirmed the pathogenicity of *A. chlamydospora* . This finding showed the implication possibility of this opportunistic pathogen for immuno-suppressed patient's infections. Occurrence of pathogenic fungi in the Liver Intensive Care Unit strongly suggests the need for regular and constant analysis of indoor air to improve the unit environment.

Keywords: Air borne fungi, *Alternaria chlamydospore*, Liver intensive care unit, rDNA, Pathology, Pathogenicity.

Introduction

Hospitals' indoor air environment can be considered a source of various microorganisms containing fungi; their adaptation characteristics favor their wide global distribution and survival in different habitats (Nevalainen et al., 2015; Coombs et al., 2018). Noble & Clayton (1963) demonstrated the contamination of hospital air by fungi, and later Lidwell & Noble (1975) reported that air conditioning devices can be a source of fungi for ambient air. Contamination of air with fungi that affect indoor air quality has been discussed in many studies (Miller., 1992; Gorny et al., 2002; Dubey., 2011; Caillaud et al., 2018). It is obvious today that most of fungal contamination of indoor air come from external air (Lee et al., 2006; Crawford et al., 2015; Abbasi & Samaei., 2018). Besides external air, domestic and everyday activities like potted plants, dog and cat hair, human skin, hair and nails, and human occupation have great impacts on increasing the concentration and diversity of fungal spores in indoor air (Reponen et al., 1992; Pitkäranta et al., 2008).

When fungal spores surround immunodeficiency patients, the risk of established nosocomial infections increases substantially (Julia et al., 2015). Measures for minimization of fungal contamination of the hospital's air environments are considered effective for controlling nosocomial infections (Gangneux et al., 2006; Pelaez et al., 2012). Contamination of indoor air by viruses, bacteria, endotoxins, pollen grains, spores of fungi and their mycotoxins are considered biological pollutants (Luengas et al., 2015; Kim et al., 2018). Temperature, moisture, and nutrient availability were found to be the main factors that led to microbes' population growth and establishment (Nazaroff, 2013).

Many types of filamentous fungi like *Cladosporium, Penicillium, Aspergillus,* and *Alternaria* sp have been isolated from contaminated air in hospitals (Okten & Asan, 2012). *Alternaria* species are filamentous pigmented (also known as dematiaceous fungi), common in the soil as saprophytes and plant pathogens and uncommonly cause infection in humans (Anaissie et al., 1989). The diseases caused by different Alternaria species include hypersensitivity, bronchial asthma, and allergies (Wiest et al., 1987; Anaissie et al., 1989). Most infections in humans are cutaneous and occur mostly in immunologic impairment patients and rarely in healthy individuals (Del Palacio et al., 1996).

This study aimed to evaluate the mycological indoor air quality of the Liver Intensive Care Unit at Suez Canal University Specialized Hospital (Egypt) and evaluate subsequent pathological effects of *A. chlamydospora* in particular as a new record isolated from   the liver intensive care unit in Egypt.

Materials and Methods

Isolation of indoor fungi
Samples collection
The present study was conducted from January 2017 to January 2018. The indoor air of the Liver Intensive Care Unit at Suez Canal University Specialized Hospital was analyzed monthly during the daytime for assessment of mycological load. Samples were collected during the first and third weeks. Both Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) media were used. The plates were open and exposed to air for 10min, and then closed and incubated at 35°C for 96hrs (Bhatia

& Vishwakarma, 2010). The average indoor temperatures during the seasons of study period were as follows: winter: 20±2°C, spring: 25 ±2°C, summer: 33 ±2 °C, and autumn: 27 ±2°C. Fans were set off during sampling (Ismail et al., 2018).

Examination of airborne fungi
After the incubation period, all the plates were examined, and fungal colonies were counted. The average colonies grown during the first and third week of each month were counted from six plates, and the numbers of CFUs/M³ air was determined using the following equation introduced by Dudzinska (2011)

$$CFU = a \times 78.6 \text{ CFU/M}^3$$

where a is the number of fungal colonies grown on the plates, CFU/M³ calculated separately for each different colony type observed on the plates. All the different air- isolated colonies were identified by their macro- and micro-morphology in regard to their color and colony texture, and then finally by microscopic investigation (De Hoog et al., 2019).

Identification of Alternaria clamydospora
DNA was extracted using the acid-washed beads extraction method (Moller et al., 1992). Fungal mycelia of *Alternaria* colony (2-3mm) was immersed in 2mL tube with glass beads containing 500μL of CTAB extraction buffer (20mM EDTA, 0.1M Tris-HCL pH 8, 1.4M NaCl, 2% CTAB and 0.1% mercaptoethanol). 20μL of the Proteinase K. solution was incubated at 56°C for 60min. After centrifugation for 3min at 12000rpm, the supernatant was transferred to a clean tube following the addition of 400μL of chloroform/isoamyl alcohol (4:1). Samples were gently mixed and centrifuged at 12000 rpm for 5min. After transferring the supernatant to a fresh Eppendorf tube, 1mL of cold absolute ethanol (-20°C) was added. Samples were kept at -80°C for an hour then centrifuged at 4°C for 10min. The liquid solution was released, and the DNA pellet was then washed with 1mL of ethanol 70% at -20°C and centrifuged at 4°C for 10min. The supernatant was discarded. Pellet was re-suspended in 50μL of TE buffer (Ultrapure water) and stored at -20°C. The quality of DNA was evaluated by electrophoresis on a 1% agarose gel.

PCR with primers ITS1/ITS2 and NS1/NS7 was applied to amplify the internal transcribed spacer ITS region and 18S rDNA, respectively.

PCR products were purified using the DNA fragment purification kit and sequenced in forward and reverse directions by Macrogen (Holland). Sequence analysis of the ITS and 18S sequences was carried out using Bio Edit Sequence Alignment Editor v.7.2.3 (Hall, 1999). Phylogenetic trees were built using the neighbor-joining (NJ) methods and bootstrap test**s.** All fungal sequences considered were at least 98% identical to the best hit in the NCBI database (Varanda et al., 2016).

Experimental animals and housing
   Animals
   Adult male and female Switzerland albino rats, weighing around 150-180 g, were obtained from the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. The animals were held at room temperature (24± 2°C) in polypropylene cages (five in each cage). Twelve hours of light and dark schedule was maintained until the animals were acclimatized to the laboratory conditions. They were fed with standard rat chow diet with *ad libitum* access to drinking water. The experiments were conducted in compliance with the guidelines of the Committee of Scientific Research Ethics at Suez Canal University (Carol et al., 2010).

Acute toxicity (LD$_{50}$) test
   Healthy albino rats of either sex (120-140g) were used for estimation of the acute toxicity (Saganuwan, 2016). The method included the use of 18 animals that were divided into six groups of three animals each. The first three animal groups were treated with a single dose of the spore suspension namely 10, 100 and 1000μL / kg; intragastrically) and the second three groups of animals were treated with higher doses (1600, 2900 and 5000μL /kg, intragastrically) of spore suspension. The general behavior and mortality of the rats were continuously monitored every 1hr after dosing during the first 24 h and then daily for the subsequent 14 days The values of 50% lethal dose (LD50) and 95% confidence were calculated using the following formula:

   LD50= √(D0 × D100).

where: D0= Highest dose that gave no mortality, D100= Lowest dose that produced mortality (Enegide et al., 2013).

In vivo pathogenicity
   A .chlamydospora spores grown in potato dextrose broth overnight were washed and suspended in sterile phosphate buffer saline and adjusted to 5 × 10^{5}CFU/mL. Rats were injected intraperitoneally with 1000μL of the fungal suspension, and then incubated for 72hrs (Segal & Frenkel, 2018).

Mode of administration and symptoms
   Twenty adult albino rats (ten females and ten males), each weighing 150-180g, were grouped as follows:

   Group (I): Male control group, treated intragastrically (IG) daily for 14 days with 500μL distilled water serving as a vehicle

   Group (II): Male infected group intraperitoneally challenged with 1000μL of the *A. chlamydospora* cells suspension with the density of 5 × 10^{5}cell/mL.

   Group (III): Female control group, treated intragastrically (IG) daily for 14 days with 500 μL distilled water serving as a vehicle.

   Group (VI): female infected group intraperitoneally challenged with 1000μL of the *A. chlamydospora* cells suspension with the density of 5 × 10^{5}cell/mL.

   After 30 days, blood samples were taken from rats before scarifying. Serum was obtained from non-heparinized blood and stored at ₋20°C for subsequent biochemical analyses (Romestaing et al., 2007). The liver, kidney, lung, pancreas, ovary, and testis tissues from different groups were dissected out, excised, and fixed in 10% formalin saline for histopathological investigation.

Blood biochemical analysis
   Under ether anesthesia, all the rats were euthanized then blood samples (2.0-4.0mL) were obtained by sinus retro-orbital puncture in tubes containing EDTA and immediately processed for hematological tests using an automatic veterinary hematological analyzer (ABC VET, HORIBA®, UK). The hematological parameters measured were mean hemoglobin (HGB), packed cell volume (PCV), count of red blood cells (RBC) and white blood cells (WBC), mean of corpuscular volume (MCV), mean of corpuscular hemoglobin (MCH), red blood cell distribution width (RDWFor biochemical analyses, 2 -3mL of blood was placed in a heparinized tube and centrifuged at 4000rpm for 15min at 4°C. The plasma obtained was stored at –20°C until use. The studied

parameters included creatinine (Creat), glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminases (GPT) were measured by the colorimetric methods described by Serap et al. (2012).

Histopathological examination

Samples of different organs were excised and fixed in 10% formalin saline, dehydrated, and then embedded in paraffin wax for microtome sectioning. The tissue sections were stained by hematoxylin and eosin (H&E) and then examined using an axiostar plus transmitted light microscope.

The histological study of kidney, heart, lung, liver, pancreas, spleen, ovary, and testis of both male and female groups were processed according to the method described by Kose et al. (2012).

Statistical analysis

The collected data were analyzed using One-way analysis of variance. The comparisons were processed using the Duncan›s multiple range test (Duncan, 1955).

Results

Differential distribution of the isolated indoor fungi

The indoor air samples collected from the Liver Intensive Care Unit at Suez Canal University Specialized Hospital were used to determine the quality of the indoor air. A total of 1661 fungal colonies containing 1601 filamentous fungi and 60 yeast cells were examined. *Cladosporium* sp. accounted to be of the highest percentage and then *Aspergillus niger, A. flavus, Penicillium* sp., *Monilia* sp., *Alternaria* sp., *Stemphyllium* sp., *A. fumigatus, Drechslera* sp., *Geotrichum* sp., *Ulocladium* sp*., Fusarium* sp., *Eurotium* sp., and *Alternaria chlamydospora* (Table 1). Identification and characterization of indoor fungi were carried out based on macro-morphology and microscopic investigation. Identification of the *A. chlamydospora* (Fig. 1) was confirmed by PCR and sequencing (accession number: AC_a (N960407)

The results revealed that population densities of indoor total fungal spores showed that their maximum spore density was scored in March, 258 CFU/m^3, followed by April, (219 CFU/m^3, and September, 162 CFU/m ^{3}. December showed the lowest spore density of 87 CFU/m (Table 1).

Analysis of the fungal bioaerosol concentration in the indoor air of the Liver Intensive Care Unit during the four seasons revealed different levels of contamination. The highest values were detected in case of *Eurotium* sp. in February, *A. niger*, *Alternaria* sp., *A. fumigatus* and *Ulocladium* sp. in March, *Alternaria* sp*., Stemphyllium* sp. and *Monilia* sp. in April, *Penicillium* sp. in May, *A. fluvus* and *Drechslera* sp. in August, *Cladosporium* sp. in September, *A. chlamydospora, Drechslera* sp., *Geotrichum* sp. in October, and *A. chlamydospora* in November. ANOVA showed that the average number of isolated colonies during the four seasons was statistically significant P<0.05 (Table 2).

Pathogenicity of Alternaria chlamydospora (in vivo model)

Blood biochemical analysis

Levels of liver and kidney enzymes (AST, ALT, and CREAT) of both the control and the *A. chlamydospora*-infected male and female groups are summarized in Table 3. The presented results revealed a significant increase in the serum AST, ALT, and creatinine in male and female infected *A. chlamydospora* groups comparing to the uninfected control counterparts.

The levels of hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, red blood cell distribution width, and total leukocyte (white blood cells) counts of both control and *"A. chlamydospora*-infected" male and female groups are summarized in Table 4 which illustrates that those parameters showed significant decreases in the infected female group in comparison to the corresponding control group. Moreover, the total leukocyte count significantly elevated in the infected female group in comparison to the corresponding control one (P<0.05). However, mean corpuscular hemoglobin and red blood cell distribution width were found not significantly different in the infected female group than in its corresponding control group. The levels of hemoglobin concentration, red blood cell count, mean corpuscular volume, and mean corpuscular hemoglobin significantly decreased in the infected male group in comparison to the control one. Additionally, the total leukocyte count was found higher in the infected male group in comparison to the control group. However, packed cell volume was not significantly different in the infected male group in comparison to the control group.

TABLE 1. Fungal spores counts and percentages (%) in one m³ air in indoor of liver intensive care unit of the Suez Canal University specialized Hospital recorded from January 2017 to January 2018 .

Isolated fungi	Jan.	Feb.	Mar.	Apr.	May	Jun	July	Aug.	Sep.	Oct.	Nov.	Dec.	Total counts (percentage)
A niger	7(3.8)%	12(6.5)%	27(14.6)%	18(9.7)%	24(13)%	24(13)%	6(3.2)%	15(8.15)%	24(13)%	12(6.5)%	9(4.8)%	6(3.2)%	184(11)%
Alternaria sp.	6(4.2)%	6(4.2)%	24(17)%	24(17)%	18(12.4)%	6(4.2)%	6(4.2)%	12(8.5)%	15(10.6)%	12(8.5)%	12(8.5)%	0	141(8.4)%
Penicillium sp.	15(8.3)%	18(10)%	30(16.6)%	24(13.3)%	30(16.6)%	18(10)%	12(6.6)%	12(6.6)%	6(3.3)%	15(8.3)%	0	0	180(10.8)%
Cladosporium sp.	12 (5)%	12(5)%	15(6.3)%	30(12.6)%	27(11.3)%	15(6.3)%	18(7.5)%	30(12.6)%	30(12.6)%	24(10.1)%	12(5)%	12(5)%	237(14.2)%
Stemphyllium sp.	7 (5)&	12(8.6)%	27(19.4)%	45(32.3)%	6(3.4)%	9(6.4)%	3(2.1)%	6(4.3)%	9(6.4)%	3(2.1)%	6(4.3)%	6(4.3)%	139(8.3)%
A.fluvus	15(8.1)%	12(6.5)%	39(21.3)%	0	6(3.3)%	9(6.4)%	12(6.5)%	21(11.4)%	24(6.4)%	15(8.1)%	6(3.3)%	24(13.1)%	183(11)%
Monillia sp.	6 (3.7)%	15(9.2)%	27(16.6)%	42(25.9)%	3(1.9)%	12(7.4)%	36(22.2)%	0	0	0	21(12.9)	0	162(9.7)%
A.fumigatus	6(4.4)%	12(8.8)%	36(26.6)%	9(6.6)%	6(4.4)%	15(11)%	6(4.4)%	6(4.4)%	15(11)%	0	9(6.6)%	15(11)%	135(8.1)%
Fusarium sp.	0	0	0	6(7.4)%	3 (3.7)%	0	6(7.4)%	9(11)%	9(11)%	0	6(7.4)%	6(7.4)%	45(2.7)%
Eurotium sp.	0	9(21.4)%	6(14.2)%	9(21.4)%	0	0	3(7.1)%	3(7.1)%	3(7.1)%	0	6(14.2)%	3(7.1)%	42(2.5)%
Ulocladium sp.	6(10.5)%	3(5.3)%	12(21)%	0	9(15.7)%	0	9(15.7)%	3(5.3)%	6(10.5)%	0	3(5.3)%	6(10.5)%	57(3.4)%
Alternaria chlamydospora	0	0	0	0	0	0	0	0	0	6(50)	6(50)	0	12(0.7)%
Drechslera sp.	6(7.1)%	6(7.1)%	12(14.2)%	0	0	3(3.5)%	6(7.1)%	12(14.2)%	9(10.7)%	12(14.2)%	9(10.7)%	9(10.7)%	84(5)%
Geotrichum sp.	3 (5)%	3(5)%	3(5)%	12(20)%	3(5)%	0	0	9(15)%	12(20)%	15(25)%	0	0	60(3.6)%
Total counts and (percentage)	89(5.3)%	120(7.2)%	258(15.3)%	219(13.1)%	135(8.1)%	111(9.5)%	123(10.5)%	138(11.8)%	162(13.9)%	114(13.9)%	105(9)%	87(7.4)%	1661

Fig. 1. *Alternaria chlamydospora* conidia (Magnification x 40)

TABLE 2. Fungal spores concentration in one m³ air in indoor of liver intensive care unit of the Suez Canal University specialized Hospital recorded from January 2017 to January 2018 (Mean±SD) CFU/m³

Isolated fungi	Jan.	Feb.	Mar.	Apr.	May	Jun	July	Aug.	Sep.	Oct.	Nov.	Dec.
A.niger	2.33+0.33c	4.33+0.33de	8.67+0.33f	6.48+0.64d	8.00+0.00g	8.33+0.33g	3.67+0.33ef	5.00+0.00e	8.00+0.00g	3.67+0.33d	2.67+0.33def	1.67+0.33bc
Alternaria sp.	2.33+0.33c	2.33+0.33c	8.33+0.33f	8.33+0.33d	6.00+0.00f	1.67+0.33b	1.67+0.33bc	3.67+0.33d	5.00+0.00f	3.67+0.33b	4.33+0.33cde	0.00+0.00a
Penicillium sp.	4.67+0.33ef	5.67+0.33f	9.33+0.33f	7.67+0.33e	9.67+0.33i	6.00+0.00f	3.67+0.33ef	3.67+0.33d	2.67+0.33d	5.33+0.33e	0.00+0.00a	0.00+0.00a
Cladosporium sp.	3.67+0.33d	3.67+0.33de	5.00+0.00e	9.67+0.33g	9.00+0.00h	5.00+0.00de	5.67+0.33g	9.67+0.33g	10.00+0.00h	8.33+0.67f	3.67+0.33gh	3.67+0.33e
Stemphyllium. sp.	2.33+0.33c	3.67+0.33de	9.00+0.58f	15.67+0.33a	3.67+0.33e	3.33+0.67c	1.00+0.00b	1.67+0.33b	3.00+0.00d	1.00+0.00b	2.00+0.00cd	2.00+0.58c
A.flavus	5.00+0.00f	4.00+0.58de	13.33+0.33g	0.00+0.00f	2.33+0.33	2.67+0.33c	4.33+0.33f	7.00+0.00f	7.67+0.33g	5.00+0.00e	1.67+0.33bc	7.67+0.33g
Monillia sp.	1.67+0.33b	4.67+0.33e	8.67+0.33f	14.00+0.58d	1.00+0.00b	4.33+0.33d	11.67+0.33h	0.00+0.00a	0.00+0.00a	0.00+0.00a	6.67+0.33i	0.00+0.00a
A.fumigatus	2.33+0.33c	3.67+0.33de	13.00+0.58f	2.67+0.33c	3.33+0.33de	5.33+0.33ef	3.33+0.33e	3.33+0.33cd	5.00+0.58f	0.00+0.00a	3.33+0.33fg	5.33+0.33f
Fusarium sp.	0.00+0.00a	0.00+0.00a	0.00+0.00a	3.33+0.33c	1.00+0.00b	0.00+0.00a	1.67+0.33bc	2.67+0.33c	2.67+0.33d	0.00+0.00a	2.00+0.00cd	2.33+0.33cd
Eurotium sp.	0.00+0.00a	3.33+0.33d	2.00+0.00c	3.00+0.00a	0.00+0.00a	0.00+0.00a	1.00+0.00b	1.00+0.00b	1.00+0.00b	0.00+0.00a	2.33+0.33cde	1.00+0.00b
Ulocladium sp.	2.00+0.00c	1.00+0.00b	4.33+0.33de	0.00+0.00a	3.00+0.00d	0.00+0.00a	3.00+0.00de	1.00+0.00b	2.00+0.00c	0.00+0.00a	1.00+0.00b	2.00+0.00c
Alternaria chlamydospora	0.00+0.00a	0.00+0.00a	0.00+0.00a	0.00+0.00a	0.00+0.00a	0.00+0.00a	0.00+0.00a	0.00+0.00a	0.00+0.00a	2.33+0.33c	2.33+0.33cde	0.00+0.00a
Drechslera sp.	2.33+0.33c	3.33+0.33d	3.67+0.33d	0.00+0.00c	0.00+0.00a	1.00+0.00b	2.33+0.33cd	3.67+0.33d	3.00+0.00d	3.67+0.33d	3.00+0.00efg	3.00+0.00de
Geotrichum sp.	1.00+0.00b	1.00+0.00b	1.00+0.00b	3.67+0.33a	1.00+0.00b	0.00+0.00a	0.00+0.00a	2.67+0.33c	4.00+0.00e	5.33+0.33e	0.00+0.00a	0.00+0.00a
P values	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

F ratio= 363.6

- Values are means ± standard error (SE).
- Means within the same row or column with different superscripts are significantly different.

TABLE 3. Toxicity of intraproteneal injections of *A. chlamydospora* (5×10^5 cell/ml) spores on rats expressed as Mean ± SE of five replicates to each group

Groups / Parameters	Cont female	Infected female	P value	Cont male	Infected male	P value	Ref. values
ALT	32.5±0.05	58.22±0.11[b]	<0.001	37.5±0.05	51.4±0.20[b]	<0.001	(5-45)
AST	69.46±0.08	126±0.57[b]	<0.001	67.4±0.07	107.6±0.11[b]	<0.001	(20-60)
CREAT	0.4±0.057	0.91±0.008[b]	<0.001	0.25±0.005	0.92±0.014 [b]	<0.001	(0.7-1.3)

- ALT: Alanine transaminase, AST: Aspartate Aminotransferase, CREAT: Creatinine.
- Values are means ± standard error (SE).
- Means within the same row or column with different superscripts are significantly different

TABLE 4. Toxicity of intraproteneal injections of A. chlamydospora)5×10^5(cell/ml on rats Hematological features. Each value is Mean ± SE of five replicates to each group

Groups / Parameters	Control female	Infected female	P value	Control male	Infected male	P value	Ref. values
HB	13.24±0.005	10.5±0.057[b]	0.001	13.66±0.088	10.3±0.057[b]	0.001	(13-18)
PCV	44.23±0.008	40.7±0.057[b]	0.001	43.1±0.057	42.733±0.088[a]	0. 11	(40-50)
RBCS	8.08±0.008	4.066±0.008[bc]	0.001	8.34±0.005	4.5±0.057[bc]	0.001	(8.5-10)
MCV	67.4±0.057	51.8±0.057[bc]	0.001	71.76±0.005	50.4±0.057[bc]	0.001	(75-100)
MCH	16.5±0.057	14.4±2.180[a]	0.579	16.5±0.057	10.5±0.057[bc]	0.014	(20-38)
RDW	23.04±0.005	23.4±0.057[a]	0. 12	22.86±0.088	23.6±0.057[a]	0.10	(20-25)
TLC x 10 ³	18±0.570	32.15±0.005[bc]	0.001	25.09±0.58	41±0.57[bc]	0.001	(10 -20)

- HB: Hemoglobin concentration, PCV: Packed cell volume, RBC: Red blood cells count, MCV: Mean corpuscular volume, MCH: Mean corpuscular Hemoglobin, RDW: Red blood cell distribution width, TLC: Total leukocyte (white blood cells) count.
- Values are means ± standard error (SE).
Means within the same row or column with different superscripts are significantly different

Changes in the histopathological features
Effects on the liver

A section of control liver (Fig. 2A) revealed a normal architecture for hepatic lobules in the portal and central areas. It was found that the *A. chlamydospora*-infected male group had many symptoms of histoplasmosis, including mild focal inflammatory cells infiltration and hydropic degeneration (HD) (Fig. 2B). Moreover, the infected females displayed mild subcapsular inflammatory cell infiltration besides cognition (CO) in the central vein and hydropic degeneration (HD) (Fig 2C).

Effects on the kidney

The control kidney (Fig. 3A) showed normal renal cortex tubules (T) and glomeruli (G). Tubules are regularly lined by cubical epithelial cells with vesicular rounded nuclei and eosinophilic cytoplasm, and glomeruli formed of capillary tufts. *A. chlamydospora*-infected males showed marked severe infiltration of inflammatory cells formed of lymphocytes and scattered neutrophils. There were areas of marked hemorrhage and replacement of tubules by congested vessels (CV), in addition to focal fibrosis with thick-walled vessels (Fig. 3B).

Effects on the lung

Sections from the lungs of the control set (Fig. 4A) showed alveoli lined by a single layer of pneumocytes (PN) with a thin alveolar wall (AV), thin-walled vessels associated with bronchioles lined by columnar epithelial cells with mucus secretion. *A. chlamydospora*-infected males showed alveoli lined by pneumocytes. There was a moderately thickened alveolar wall with moderate focal inflammatory infiltrate formed mainly of lymphocytes (Fig. 4B). *A. chlamydospora*-infected females showed alveoli lined by pneumocystis associated with a moderately thickened alveolar wall with moderate focal inflammatory infiltrate formed mainly of lymphocytes (Fig. 4C).
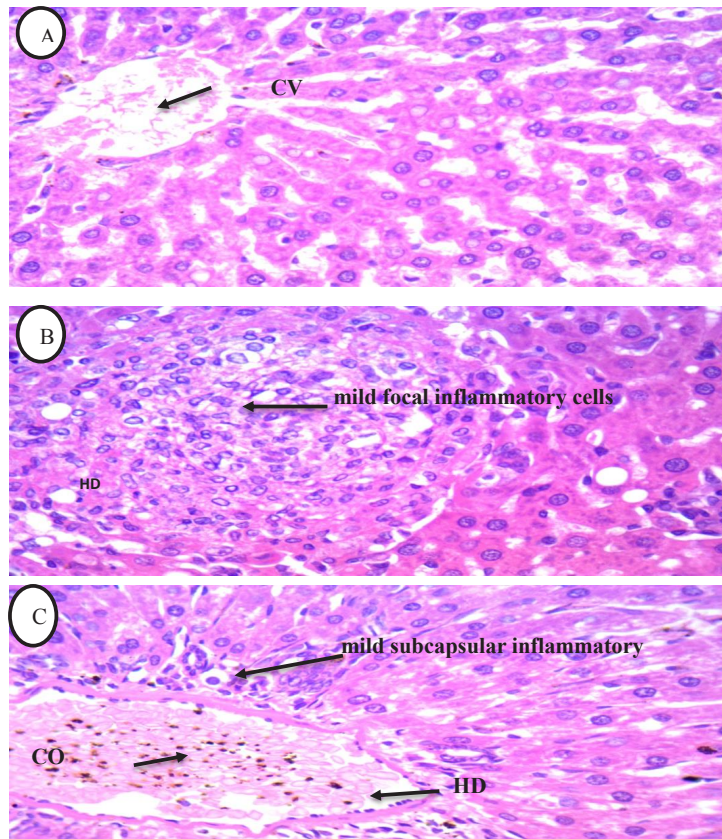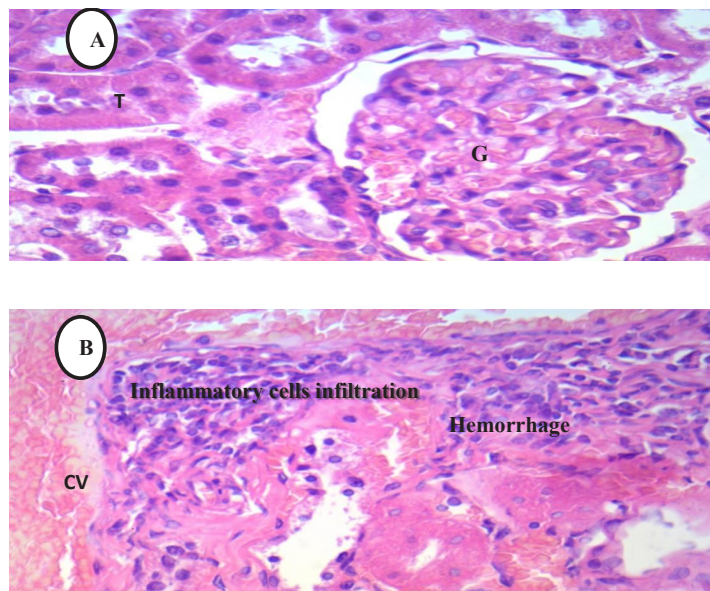
Fig. 2. Histopathological findings in central area of rat liver [(A) Control displayed normal morphology of the classic hepatic lobule. At the center of the lobule is the central vein (CV); (B) "*A. chlamydospora* infected" males exhibited mild focal inflammatory cells infiltration besides hydropic degeneration (HD). (C) "*A. chlamydospora* infected" females exhibited mild subcapsular inflammatory cell infiltration besides cognition (CO) in the central vein and hydropic degeneration (HD). Magnification (X200)]

Fig. 3. Histopathological findings in kidney [(A) Control showed renal cortex tubules (T) and glomeruli (G), (B) "*A. chlamydospora* infected" males showed marked severe inflammatory cells infiltration, there was areas of marked hemorrhage and replacement of tubules by congested vessels (CV). Magnification (X200)]
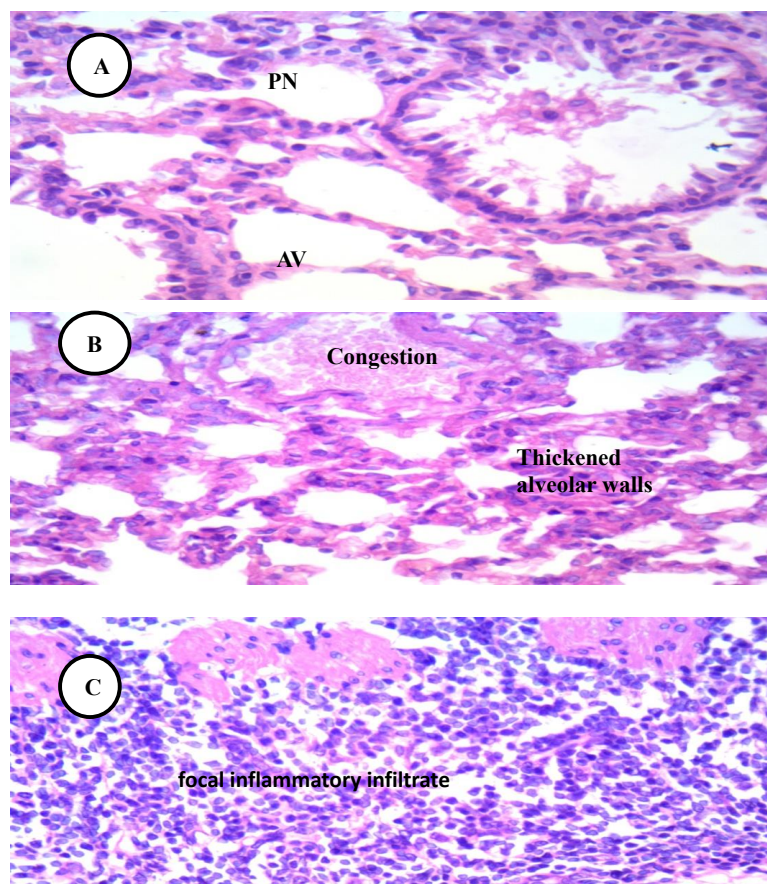
Fig. 4. Histopathological findings in lung **[(A) control section showing alveoli lined by a single layer of PN pneumocytes, with a thin alveolar wall (AV), (B) "*A. chlamydospora* infected" males; there was moderately thickened alveolar walls with moderate focal inflammatory infiltrate, (C) "*A. chlamydospora* infected" female; there was moderately thickened alveolar walls with moderate focal inflammatory infiltrate. Magnification (X200)]**

Effects on the pancreas

Sections in pancreatic tissue of the control individuals (Fig. 5A) found formed of acinar cubical cells with basophilic nuclei and eosinophilic cytoplasm, arranged in acini separated by a thin fibrovascular stroma. *A. chlamydospora*-infected males showed mild edema and congestion (Fig. 5B), while *A. chlamydospora*-infected females showed few dilated vessels (Fig. 5C).

Effects on the heart

Sections in heart muscle of the control groups (Fig. 6A) showed bundles and fascicles of cardiac muscle cells with oval nuclei and preserved myofibrils, separated by thin fibrovascular septa. *A. chlamydospora*-infected males showed moderate edema and moderately- congested vessels with focal hemorrhage (Fig. 6B).

Effects on the testis

Sections in control testicular tissue (Fig. 7A)

showed seminiferous tubules with a thin basement membrane lined by germ cells at different stages of maturation, while the lumen showed scattered spermatozoa. *A. chlamydospora*-infected males showed degenerated germ cells with dilated seminiferous tubules. (Fig. 7B).

Effects on the ovary

Histological observation of ovarian tissues (Fig. 8A) showed numerous healthy follicles at various stages of development and new corpus luteum. Most of these follicles revealed normal features expressed by intact oocytes, absence of pyknotic granulosa cells, absence of fragmented granulosa cells and cell debris in the antral cavity. *A. chlamydospora*- infected females showed cellular spindle stroma with the cortex, scattered primordial follicles, and maturing follicles. There were a few corpora luteal (Fig. 8B).
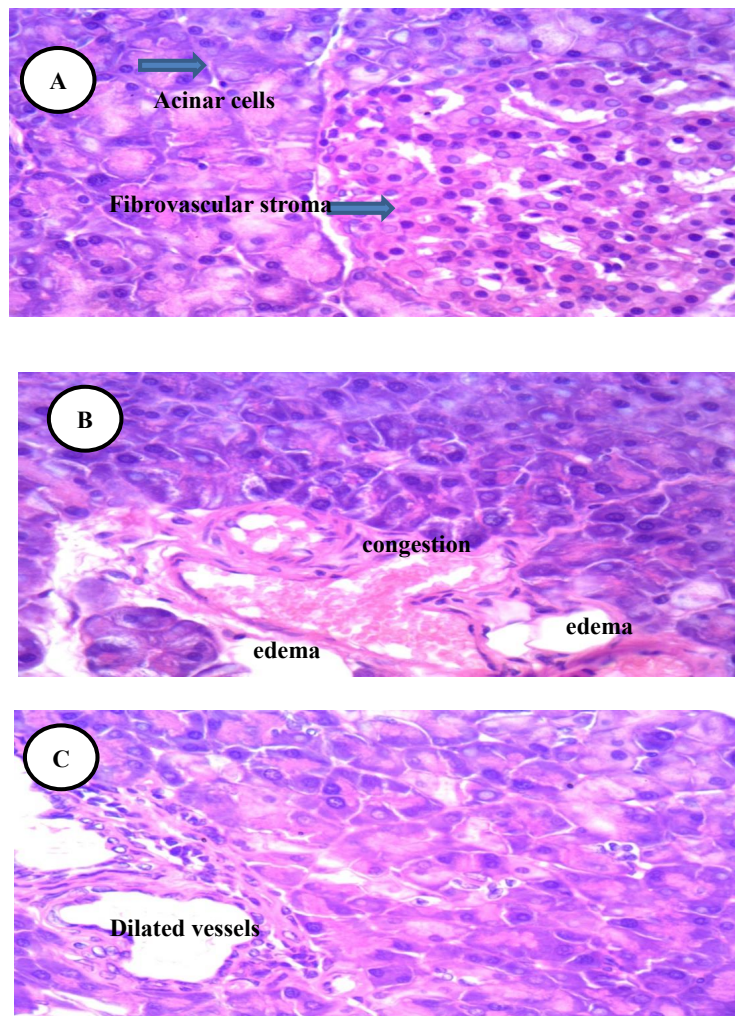
Fig. 5. Histopathological findings in the Pancreas [(A) control pancreatic tissue formed from acinar cubical cells, (B) "*A. chlamydospora* infected" males exhibited mild edema and congestion, (C) "*A. chlamydospora* infected" females showed few dilated vessels. Magnification (X200)]
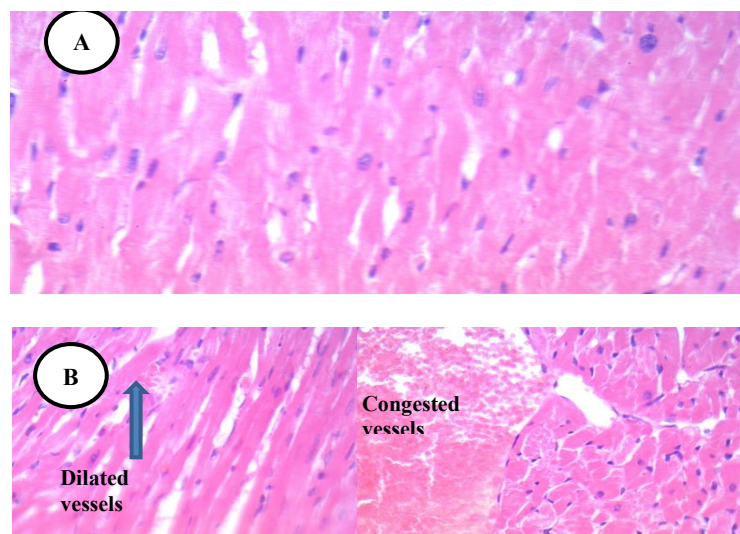

Fig. 6. Histopathological findings in heart [(A) control, (B) "*A. chlamydospora* infected" malse showed moderate edema and moderately congested vessels with focal hemorrhage. Magnification (X200)]
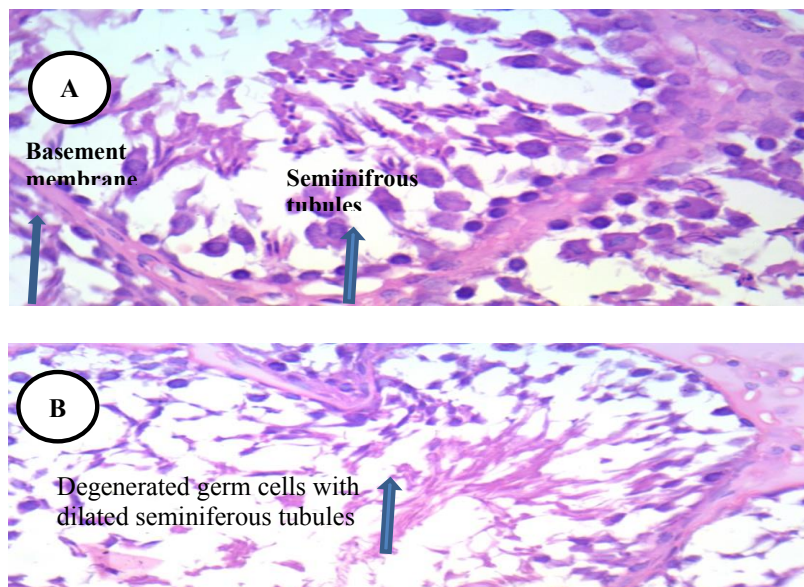
Fig. 7. Histopathological findings in testis [(A) control, (B) "*A. chlamydospora* infected" male showed degenerated germ cells with dilated seminiferous tubules Magnification (X200)]
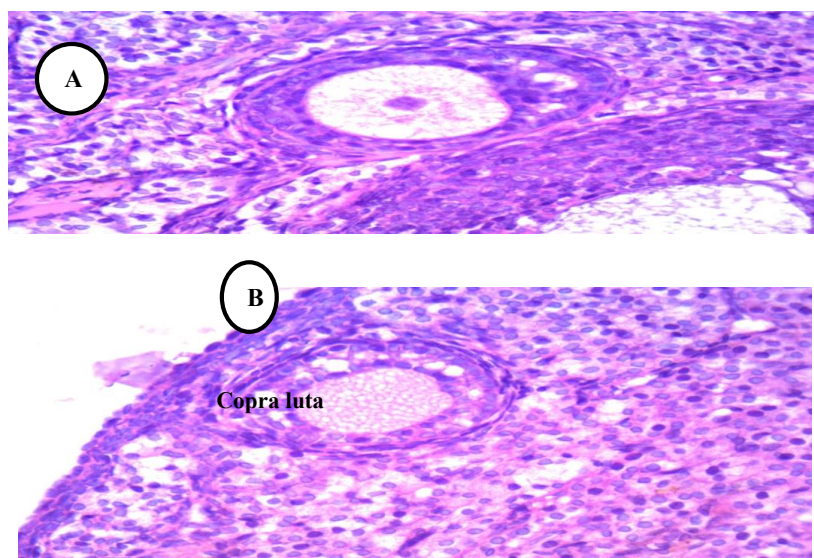

Fig. 8. Histopathological findings in ovary [(A) control, (B) "*A. chlamydospora* infected" female showed cellular spindle stroma with cortex scattered primordial follicles and maturing follicles. There were few corpora lutes. Magnification (X200)]

<u>**Discussion**</u>

In the present study, different types and percentages of fungi were isolated from the indoor air of the Liver Intensive Care Unit at Suez Canal University Specialized Hospital during a whole year, confirming the previous documented results (Krajewska-Kulak et al., 2009; Sautour et al., 2009; Afshari et al., 2013; Sepahvand et al., 2013; El-Sharkawy & Noweir, 2014; Cabo Verde et al., 2015; Godini et al., 2015; Eslami et al., 2016).

The study revealed that the highest percentage of isolated fungi was of *Cladosporium sp* (14.2%), followed by *Aspergillus niger* and *A. flavus* (11%), and then by *Penicillium* sp. (10.8%). These findings were also documented by other research teams in Iran. Furthermore, Kim et al. (2010) expressed that the genera *Cladosporium, Penicillium* and *Aspergillus* were the most dominant air isolated species**.** It is important to note that because of the small size (< 2mm) of *Cladosporium* sp. spores, they remain suspended

in the air for a long time which increases their existence and willing for inhalation (Keyvan et al., 2007).

The present findings match with the findings of Aboul-nasr et al. (2014) who recorded that the most frequent airborne fungi contaminating intensive care units and operation rooms in Assuit hospitals, Egypt, were *Cladosporium, Aspergillus, Penicillium* and *Fusarium.* Additionally, Rangaswamy et al. (2013) found that *Aspergillus* sp.*, Alternaria* sp., *Penicillium* sp., and *Fusarium* sp. were the most frequent in health care centers in India.

Previous studies recorded that *spergillus sp* was the most prevalent cause of nosocomial fungal infections (aspergillosis) among patients who were immunocompromised and those at high risk (Perlroth, 2007; Ostrosky-Zeichner, 2008; Bajwa & Kulshrestha, 2013; Souza et al., 2019). The high distribution of fungi in the hospitals' indoor air results in high mortality rates, especially in immune-compromised patients (El-Sharkawy & Noweir, 2014; Afanou et al., 2015).

During this study, different species of *Aspergillus* were isolated. Cristina et al. (2009) and Boff et al. (2013) described them as "pathogenic". They suggested the possibility of development of various symptoms that can lead to illness in lung as well as an emerging risk factor in immunocompromised patients, neonates and children. In this study, a new record of *A. chlamydospora* obtained from indoor air of the liver intensive care unit under investigation showed significant pathogenic effects by increasing some parameters containing liver and kidney enzymes in addition to its pathogenic effects in the different visceral organs of rats. This study accorded with the findings of Pastor & Guarro (2008) who reported that *A. chlamydospora*, along with other *Alternaria* species, are saprophytes dematiaceous ascomycete fungi with worldwide distribution. It is an opportunistic human pathogen that is frequently associated with allergic respiratory diseases causing hay fever or hypersensitivity reactions that sometimes lead to asthma in immunocompromised hosts. Later, Dedola et al. (2010) reported that Alternariais is one of the Phaeohyphomycoses pigmenters fungi present worldwide in a variety of environments and climates and common in soil as a saprophyte or present as a plant pathogen. However, the findings

of the present study matched also those of Naidu et al. (2000) who detected that different visceral organs of rats, infected by *A. chlamydospora* up to 30 days post-infection, expressed mixed inflammatory reactions in liver, brain, kidneys, lungs, and stomach disclosing serious effects in immuno-suppressed patients.

Conclusion

In conclusion, the results generated in the current study demonstrated that the indoor air of the Liver Intensive Care Unit contained a high diversity of fungal species. These fungi may lead to infections in this unit. Therefore, hospitals should have enhanced the practice of good hygiene protocols to limit microbial concentration load in the air and avoid infections. Routinely monitoring the hospital mycoflora is urgently recommended. Additionally, taking care of personal hygiene is the most effective measure to avoid nosocomial infections.

Compliance with ethical standards: The study was achieved in accordance with the ethical standards of the institutional research committee (Carol et al., 2010).

Statement on the welfare of animals: Rats' experiments were conducted in compliance with the guidelines of the Committee of Scientific Research Ethics in Suez Canal University approved under the number 2018005. All applicable institutional guidelines for the care and use of animals were followed.

Conflict of interest: The authors declare no conflict of interest

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إحداث المرض في جسم الكائن الحي لفطر الألترناريا كلاميدوسبرا   المعزوله من الهواء الداخلى لوحده العنايه المركزه للكبد

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تتأثر جودة الهواء فى البيئة الداخلية بوجود كائنات دقيقة مثل الفطريات والبكتيريا. تم تصميم هذا العمل لتقييم جودة الهواء الداخلي في وحدة العناية المركزة للكبد في مستشفى جامعة قناة السويس التخصصي، مصر، على مدار عام واحد باستخدام الطرق التقليدية. تم عزل أربعة عشر نوعًا من الفطريات وتم تحديدها على أنها *Aspergillus niger* و *Penicillium* sp. و *Alternaria* sp. و *Cladosporium* sp و *Stemphyllium* sp و. تم تحديد وتعريف ريكورد جديد معزول من الهواء، وهو *Alternaria chlamydospora* ، باستخدام التتابع الجينى للكائن. وقد تم دراسه تأثيره الممرض على ذكور وإناث الجرذان البيضاء. لذلك تم عمل العديد من تحاليل الدم مثل الهيموجلوبين (HGB) ، حجم الخلايا المضغوطه (PCV) ، خلايا الدم الحمراء (RBC) ، متوسط حجم الخليه (MCV) ، خلايا الدم البيضاء (WBC) ، متوسط الهيموجلوبين (MCH) ، خلايا الدم الحمراء تم قياس توزيع كريات الدم الحمرا (RDW) والعدد الكلي للكريات البيضه (TLC). بالإضافة إلى ذلك، تم تقدير الكرياتينين (Creat) ، ومستوى انزيم ناقلة أمين الأسبارتات (GOT) ومستوى إنزيم ناقلة أمين الألانين (GPT) لقد تم عمل هستولوجى للأعضاء المختلفه للجرذان البيضاء حتى يتم تقدير مدى القدره المرضيه للفطر محل الدراسه .