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**Original Research Article**
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# PHENOTYPIC CHARACTERIZATION OF SOME PATHOGENIC FUNGI ISOLATED FROM POULTRY AND THEIR SURROUNDINGS IN EL–GHARBIA GOVERNORATE, EGYPT

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**Abstract:** Poultry production is affected by several fungal diseases. Such fungal infection occurs in poultry farms via using a moldy litter, or ingestion of contaminated drinking water or moldy ration. In this study, a total of 210 birds with a history of respiratory distress of different breeds were collected randomly from sporadic different private farms and hatcheries in El–Gharbia Governorate, Egypt. The birds were sacrificed, then a total of 1050 tissue specimens from lung, air sacs, liver, crop and trachea were collected. In addition, 40 samples of poultry ration, 14 bedding materials, 4 air samples and 29 water samples were also collected. The collected samples were cultured on Sabouraud's agar plates. Macromorphological and micromorphological fungal examinations were performed for phenotypic characterization. Histopathological examinations were also performed using with hematoxylin and eosin stains. Antifungal sensitivity testing was screened using Mueller's Hinton Agar for studying the susceptibility of the recovered fungal isolates to the most commonly used antifungal drugs in Egypt, namely amphotericin B, clotrimazole, fluconazole, itraconazole, ketoconazole, and nystatin. The obtained results demonstrated that mold isolation was the highest in the collected samples from birds at 36.84%, followed by drinking water (31.57%). The highest incidence of mold isolation was recorded at the lungs of broilers and baladi birds followed by the air sacs. While in saso birds, the highest incidence was at the air sac. Collectively, 97 mold strains were identified from the lung, 74 from the air sacs, 30 from the liver, 61 from the trachea, and 44 from the crop. In addition, 19 mold isolates were recovered from the bird surroundings. *Aspergillus niger* as well as *Penicillium chrysogenum* were recovered and showed resistance to ketoconazole, while *Cladosporium perangustum* was resistant to fluconazole. All of the isolated molds were sensitive to itraconazole and nystatin except *A. flavus* that was resistant to nystatin. All *Aspergillus spp.* were resistant to fluconazole except *A. niger.* In conclusion, *Aspergillus spp.* was the most associated mold with poultry species and their surroundings in Egypt farms. Itraconazole and nystatin could be applied as proper antifungal drugs the control of for *Aspergillus* infection in birds.

**Key words:** phenotypic; fungi; poultry; antifungal sensitivity

## Introduction

The poultry industry has grown at a rate of more than 5% annually over the past three decades, and its proportion of global meat production has also expanded from 15% to 30% at the moment (1). One of the primary agricultural sectors in Egypt is the poultry sector, which has received over 18 billion LE in investment. There are over 1.5 million permanent employees and roughly 1 million temporary employees in the labor market. The sector contributes significantly to the nation's supply of animal protein (white meats and eggs) (2).

Poultry production is affected by several fungal diseases. Due to the abuse of broad spectrum antibiotics, corticosteroids, and immune-suppressive medications, as well as the rise in the number of patients who are terminally ill and unwell, a fungal infection has recently become a global health concern and a significant cause of respiratory infections in poultry. Fungi are heterotrophic eukaryotes that can be single cells

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or multicellular. They obtain their sustenance through nutrient absorption and can reproduce asexually, sexually, or both (3).

The source of infection to poultry farms occur either via using of a moldy litter or from hatcheries when one day-old bird have retained conidia arrived at the farms. Other sources of farm contamination might occur through poor quality feed stuffs, improper bedding management, the entrance of air loaded with conidia. In poultry farms, humidity, and temperature conditions stimulate the hyphae growth and multiplication of fungi leading to the huge production of conidia, which are subsequently spread and inhaled by the birds (4).

Through the creation of mycotoxins, which have the potential to inflict economic losses, fungal illnesses result in a direct or indirect infection. Some fungi are pathogenic for birds, and they also cause fatal diseases in humans (5). The fungi cause certain pathological changes in the host, including inflammation, lesions, and illness that can result in death. It mostly affects the neurological and respiratory systems of chickens. Their saprophyte lifestyle results in propagation and dispersion; infection is a dead end, with the exception of favus (Dermatophytosis), as mycoses are not contagious (3).

Aspergillosis, candidiasis, favus, and cryptococcosis are examples of fungal illnesses that affect chicken farms. Additionally, both aspergillosis and candidiasis were the most significant ones. These species are frequently discovered in soil, decomposing organic matter, animal feed, dried grains, and others. Additionally, fungal species are in charge of the biodegradation of materials, the rotting of food and beverages, and the production of harmful mycotoxins. This attracts the attention for investigating the source and reservoir of those fungi (6).

It was reported that symptoms of aspergillosis depend on the organs affected and whether infection is localized or disseminated. Aspergillosis appears to be more significant in confinement situations where stress factors may be involved or where moldy litter or grain is present. *Aspergillus fumigatus* is considered as a major pathogen in birds. Other species like *A. flavus*, *A. niger, A. nidulans*, and *A. terreus* may also be isolated from avian cases of aspergillosis (sometimes in mixed infections) but much less frequently than *A. fumigatus* (2).

Traditional microscopic and cultural features are still often utilized to identify fungi. Conidial and mycelial color, colony reverse color, colony diameter, soluble pigments, and exudate production are used to make macro-morphological identification. Microscopic identification depends primarily on seriation, vesicle shape and size, conidia and stipe morphology (7).

The aim of this study was to identify the phenotypic characteristics of some fungi isolated from poultry and their surroundings.

## Materials and methods

### *Sample collection*

A total of 210 birds with a history of respiratory distress were collected randomly from broiler chicks (n = 180), Baladi breeds (n = 5), and Saso chickens (n = 25) from sporadic different private farms and hatcheries in El–Gharbia Governorate, Egypt. These farms showed different mortality rates during the period from November 2020 to July 2021. The birds were sacrificed; out of 1050 tissue specimens from lung, air sacs, liver, crop and trachea were collected under complete aseptic condition. Besides, a total of 87 samples of the surroundings of the poultry were collected (40 samples of poultry ration, 14 bedding materials, 4 air samples and 29 water samples).

### *The mycological examination*

The prepared samples were cultured on to Sabouraud's Dextrose agar plates with a sterile glass rod. The cultured plates were incubated at 25°C and examined daily for five days. Mould colonies were picked up and subcultured on slope agar for further identification. The mould cultures were purified by sub-culturing on Sabouraud's agar plates, incubated at 25°C for 3-5 days and examined for macro and micromorphological characters.

Samples for histopathological examination originated from lung nodules of the affected birds were collected for histopathological examination (8).

#### Preparation of sample homogenates according to (9)

The sacrificed birds were aseptically eviscerated and each sample was taken directly to a sterile blender containing 90 ml of peptone water. Then ten grams from each sample (lung, liver, trachea, air

sacs, and crop) were aseptically placed in a sterile blender with 90 ml of 0.1% sterile peptone water.

For the bedding material and poultry ration samples

The mixture was homogenized for three minutes at 2500 rpm using sterile homogenizer (Type M-P3-302, Mechanic, Precyzina, Poland), then allowed to stand 2 minutes at room temperature and the contents were mixed by shaking before applying to the media.

Colony count of fungal cell in ration samples

Serial dilutions ($10^{-1}$:$10^{-8}$) were made by adding 1 g of each feed sample to 9 ml of sterile physiological saline (10). One milliliter was inoculated onto Sabouraud's Dextrose Agar (SDA) using spread plate method and incubated at 35 °C for 3–7 days. Single colonies of fungal growth were identified on the basis of morphologically and microscopically by using the stain lacto phenol cotton blue (11).

For air sampling

using passive sampling technique as described (12), the sterilized labelled plates of SDA were taken to the farms with history of bad condition of ventilation, all the openings of the farm were closed (for indoors result), air flow was allowed, then open in each corner of the farm the plates were opened and left for a few minutes then closed and placed in tightly closed sterilized plastic bag then transported to the lab under aseptic condition for incubation at 25°C for five days and examined daily.

For water sampling

by using a sterile syringe 100 ml from each sample were taken and placed in a sterile cooled container for delivery to the laboratory without delay, and directly 10 ml of the sample was added to 90 ml of peptone water 1%, sub-samples of 0.1 ml were then plated and spread evenly on to SDA plates and incubated at 25°C for five days and examined daily for identification and enumeration (direct plating technique (13).

*Isolation of the mould genera from examined poultry samples*

From the previously diluted poultry-originated samples, 0.1 ml was spread evenly on to SDA. The inoculated plates were incubated at 25°C and examined daily for five days. Colonies were picked up and subcultured on slope agar for further identification.

*Mould identification*

The mould cultures were purified by sub-culturing on Sabouraud's agar plates, incubated at 25°C for 3-5 days and examined for macro and micromorphological characters (14) as follows:

Macromorphological characters

The morphological examination included rate of growth, texture changes in color of the surface and reverse sides of the cultures.

Micromorphological characters

A small portion of the periphery of fresh culture was picked and placed on a clean slide with a drop of lactophenol cotton blue stain and examined microscopically to detect septation of hyphae, roughness or smoothness of conidiophores, shape of the vesicles, arrangement and number of the rows strigmata.

*Histopathological examination*

Samples for histopathological examination originated from lung nodules of the affected birds were collected from different groups, then fixed in 10% neutral buffered formalin. After dehydration and clearance, the tissues were embedded in paraffin and sectioned in 5 μm thickness. The serial sections were subjected to staining with hematoxylin and eosin (8).

*Antifungal sensitivity test*

Plates were prepared with Mueller Hinton Agar, modified according to CLSI guides for antifungal sensitivity testing using antifungal discs. Sterile non-toxic cotton swabs were dipped into the standardized inoculum. The entire agar surface of the plate was streaked with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5-15 minutes with lid in place. The discs were applied using a septic

technique. Deposit the discs with centers at least 24 mm apart. The plates were inverted and place in an incubator set to 35°C within 15 minutes after the discs were applied. Each plate was examined after 20-24 hours of incubation. The results were read at 48 hours only when insufficient growth was observed after 24 hours. Six antifungal discs were used; Amphotericin-B, Clortrimazole, Fluconazole, Itraconazole, Ketoconazole, and Nystatin.

## Results

### *Incidence of moulds in poultry samples*

From 210 birds with 1050 tissue pieces, 306 fungal spp. were isolated and identified, as showed in Table 1. The highest incidence was in lungs among the broiler and Baladi birds followed by the air sacs then trachea. In Saso birds, the highest incidence was in air sacs followed by the lungs then the trachea.

Total number and percentage of moulds isolated from lung samples

As shown in Table 2, the mycological examination of 210 lung samples revealed the isolation of 97 strains of mould spp., which were identified as (*Aspergillus spp.,* and *Peniciillium spp.*). The highest incidence was for *A. flavus* (50.5%) followed by *Penicillium chrysogenum* (23.71%).

Total number and percentage of moulds isolated from air sacs samples

As shown in Table 3, the mycological examination of 210 air sac samples revealed the isolation of 74 mould stains were identified as (*Aspergillus spp.* and *Peniciillium spp.)* The highest incidence was for *A. flavus* (43.24%) followed by *Penicillium chrysogenum* (25.68%).

Total number and percentage of moulds isolated from liver samples

As showed in Table 4, from 210 liver samples, 30 mould stains were identified as (*Aspergillus spp.*, and *Penicillium spp.*). The highest incidence was for *Penicillium chrysogenum* (40%)., followed by *A. flavus* (36.66%).

Total number and percentage of moulds isolated from trachea samples

As showed in Table 5, from 210 trachea samples, 61 mould stains were identified as (*Aspergillus spp.*, and *Penicillium spp.*). The highest incidence was for *A. flavus* (40%) followed by *A. niger* (27.87%).

Total number and percentage of moulds isolated from crop samples

As showed in Table 6, from 210 crop samples 44 mould strains were identified as *Aspergillus spp.*, and *Peniciillium spp.* The highest incidence was for *Penicillium chrysogenum* (65.9%), followed by *A. flavus* (25%)

### *Incidence and percentage of mould isolated from the surroundings of poultry*

Mycological examination of 87 surroundings samples revealed the isolation of 19 strains of mould spp. as shown in Table 7.

### *Total number and percentage of moulds isolated from ration samples*

From 40 ration samples, 7 mould isolates were identified (*Aspergillus spp., Penicillium spp.,* and *Cladosporium spp.)* as shown in Table 8.

### *Total number and percentage of moulds isolated from beddings samples*

From 14 bedding material samples, 4 mould isolates were identified (*Aspergillus spp.* and *Penicillium spp.)* as shown in Table 9.

### *Total number and percentage of moulds isolated from air samples*

From 4 air samples, 2 mould isolates were identified (*Aspergillus spp.* And *Penicillium spp.*) as shown in Table 10.

### *Total number and percentage of moulds isolated from water samples*

From 29 water samples, 6 mould isolates were identified (*Aspergillus spp*) as shown in Table 11.

**Table 1:** Incidence of moulds isolated from the poultry

| Type of birds | Broiler (180) | | Baladi (5) | | Saso (25) | |
|---|---|---|---|---|---|---|
| Organ | No. of +ve | % | No. of +ve | % | No. of +ve | % |
| Lung | 77 | 25 | 3 | 0.9 | 17 | 5.5 |
| Air sac | 52 | 16.44 | 3 | 0.9 | 19 | 6.2 |
| Liver | 21 | 6.86 | 1 | 0.3 | 8 | 2.6 |
| Trachea | 44 | 14.37 | 2 | 0.6 | 15 | 4.4 |
| Crop | 32 | 10.4 | 2 | 0.6 | 10 | 3.2 |
| Total strains | 306 | | | | | |

*The percentage of +ve samples = the numbers of +ve strain samples divided on the total numbers of +ve strains multiplied by 100.

**Table 2:** Total No. and percentage of fungi isolated from lung samples

| Mould Species | No. of +ve isolates | % | Total No. of examined samples |
|---|---|---|---|
| *A. flavus* | 49 | 50.5 | 210 |
| *A. fumigatus* | 13 | 13.4 | |
| *A. niger* | 12 | 12.37 | |
| Total *Aspergillus* species | 74 | 76.24 | |
| *Penicillium chrysogenum* | 23 | 23.71 | |

*The Percentage of +ve isolates calculated according to total number of the +ve isolates of all mould strains isolated from lung (97).

**Table 3:** Total No. and percentage of fungi isolated from air sacs samples

| Mould Species | No. of +ve isolates | % | Total No. of examined samples |
|---|---|---|---|
| *A. flavus* | 32 | 43.24 | 210 |
| *A. fumigatus* | 11 | 14.86 | |
| *A. niger* | 12 | 16.22 | |
| Total *Aspergillus species* | 55 | 74.32 | |
| *Penicillium chrysogenum* | 19 | 25.68 | |

*Percentage of +ve isolates calculated according to total number of the +ve isolates of all mould strains isolated from air sacs (74).

**Table 4:** Total No. and percentage of fungi isolated from liver samples

| Mould Species | No. of +ve isolates | % | Total No. of examined samples |
|---|---|---|---|
| *A. flavus* | 11 | 36.66 | 210 |
| *A. fumigatus* | 2 | 6.66 | |
| *A. niger* | 5 | 16.66 | |
| Total *Aspergillus species* | 18 | 59.98 | |
| *Penicillium chrysogenum* | 12 | 40 | |

*The Percentage of +ve isolates calculated according to total number of the +ve isolates of all mould strains isolated from liver (30).

**Table 5:** Total No. and percentage of fungi isolated from Trachea samples

| Mould Species | No. of +ve isolates | % | Total No. of examined samples |
|---|---|---|---|
| *A.flavus* | 25 | 40 | 210 |
| *A.fumigatus* | 7 | 11.48 | |
| *A.niger* | 17 | 27.87 | |
| Total *Aspergillus species* | 49 | 79.35 | |
| *Penicillium chrysogenum* | 12 | 19.67 | |

*The Percentage of +ve isolates calculated according to total number of the +ve isolates of all mould strains isolated from trachea (61).

**Table 6:** Total No. and percentage of fungi isolated from crop samples

| Mould Species | No. of +ve isolates | % | Total No. of examined samples |
|---|---|---|---|
| *A. flavus* | 11 | 25 | 210 |
| *A. fumigatus* | 2 | 4.55 | |
| *A. niger* | 2 | 4.55 | |
| Total *Aspergillus species* | 15 | 34.09 | |
| *Penicillium chrysogenum* | 29 | 65.9 | |

*The Percentage of +ve isolates calculated according to total number of the +ve isolates of all mould strains isolated from trachea (44).

**Table 7:** Total No. and percentage of fungi isolated from surroundi

| | No. of +ve samples | %of +ve samples | Total number of samples(87) |
|---|---|---|---|
| Air | 2 | 10.52 | 4 |
| Bedding | 4 | 21 | 14 |
| Ration | 7 | 36.84 | 40 |
| Water | 6 | 31.57 | 29 |
| Total strains | 87 | | 19 |

*The percentage of +ve samples = the numbers of +ve samples divided on the total numbers of +ve strains multiplied by 100.

**Table 8:** Total No. and percentage of fungi isolated from ration samples

| Mould Species | No. of +ve isolates | % of +ve isolates | Total No. of ration samples |
|---|---|---|---|
| *A. fumigatus* | 2 | 28.57 | 40 |
| *A. niger* | 2 | 28.57 | |
| *P. chrysogenum* | 2 | 28.57 | |
| *Cladosporium* | 1 | 14.29 | |

*The Percentage of +ve isolates calculated according to total number of the +ve isolates of all mould strains isolated from ration (7).

**Table 9:** Total No. and percentage of fungi isolated from bedding material samples

| Mould Species | No. of +ve isolates | % | Total No. of bedding material samples |
|---|---|---|---|
| *A. flavus* | 2 | 50 | 14 |
| *A. niger* | 1 | 25 | |
| *P. chrysogenum* | 1 | 25 | |

*The Percentage of +ve isolates calculated according to total number of the +ve isolates of all mould strains isolated from bedding materials (4).

**Table 10:** Total No. and percentage of fungi isolated from air samples

| No. of +ve isolates | Mould Species | % | Total No. of air samples |
|---|---|---|---|
| 1 | *A. flavus* | 50 | 4 |
| 1 | *P. chrysogenum* | 50 | |

*The Percentage of +ve isolates calculated according to total number of the +ve isolates of all mould strains isolated from air (2).

**Table 11:** Total No. and percentage of fungi isolated from water samples

| No. of +ve isolates | Mould Species | % | Total No. of water samples |
|---|---|---|---|
| 4 | *A. flavus* | 66.6 | 29 |
| 2 | *A. fumigatus* | 33.3 | |

*Percentage of +ve isolates calculated according to total number of the +ve isolates of all mould strains isolated from water (6).

**Table 12:** Total mould count of the examined ration and drinking water samples:

| Type of sample | No. of examined samples | % of +ve samples | Min. mould count (cfu\g or 100 mL) | Max. mould count (cfu\g or 100 mL) | Mean count (cfu\g or 100 mL) | Standard error |
|---|---|---|---|---|---|---|
| Ration | 40 | 17.5 | 6× | 12 | $1.01\times10^{7}$ | $4.6\times10^{6}$ |
| Drinking water | 29 | 20.68 | 12 | 118 | 30.6 | 7.8 |

**Table 13:** Antifungal susceptibility testing of the recovered mould species

| Mould Species | AmP | CLC | FLC | ITC | KTC | NYT |
|---|---|---|---|---|---|---|
| *P. Chrysogenum* | S | S | S | S | R | S |
| *Cladosporium perangustum* | S | S | R | S | S | S |
| *P. Chrysogenum* | S | S | S | S | R | S |
| *A. flavus* | R | S | R | S | R | R |
| *A. flavus* | S | R | R | S | R | S |
| *P. chrysogenum* | S | S | S | S | S | S |
| *P. chrysogenum* | S | S | S | S | R | S |
| *A. flavus* | S | R | R | S | S | R |
| *A. niger* | S | S | S | S | R | S |

AmP: Amphotericin B (100 µg/disc), CLC: Clotrimazole (10 µg/disc), FLC: Fluconazole (100 µg/disc), ITC: Itraconazole (10 µg/disc), KTC: Ketoconazole (100 µg/disc), NYT: Nystatin (10 µg/disc), S: sensitive, R: resistant.

### *Histopathological findings*

Nodular lesions on lung and pleural surface were examined. The examined nodules showed central massive liquifactive necrosis involving wide area of the alveolar and bronchial tissues with presence of hyphal elements, with characteristic basophilic septated hyphae, with about 45 angle degree of branched manner, which might be consistent with Aspergillosis (Figures 1-4).

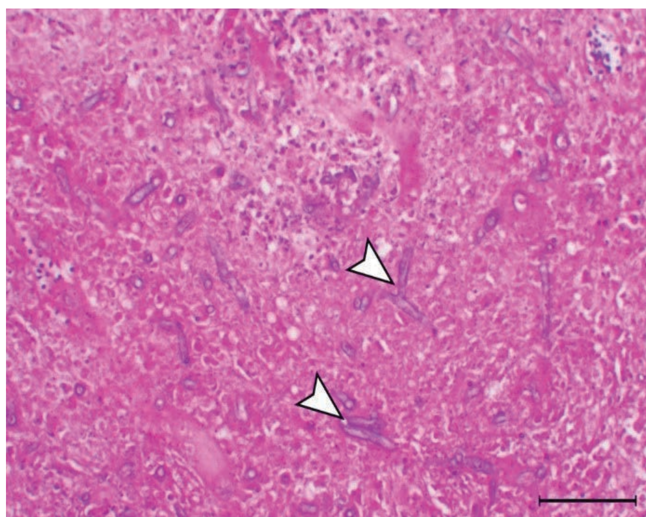

**Figure 1:** The lung of infected bird with *Aspergillosis* showing nodule associated with liquifactive necrosis with presence of branched hyphae (arrowheads). H&E, X200, bar= 50 µm

### *Total mould count of the examined ration and drinking water samples:*

The obtained result revealed that the total mould count in poultry feed samples ranged from 6 x $10^{3}$ to 12 x $10^{7}$cfu/g and this indicated that the ration is highly contaminated with mould, on the other hand it was found that the total mould count of the examined drinking water ranged from 12 to 118 cfu/100 ml and also this indicates that

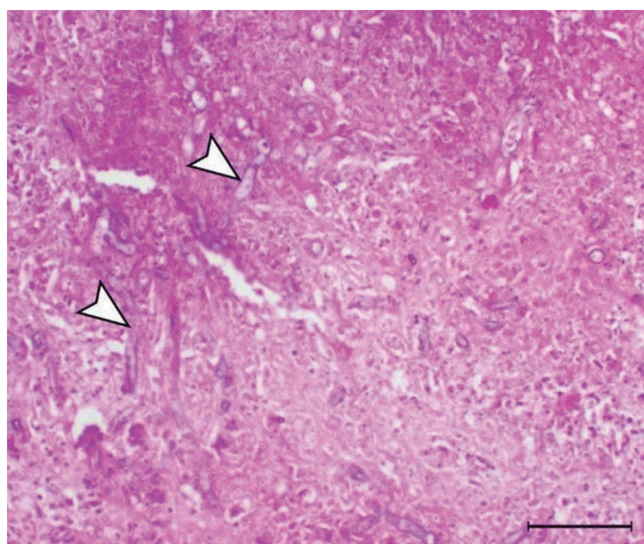

**Figure 2:** The lung of infected bird with Aspergillosis showing basophilic septated hyphae (arrowheads). H&E, X200, bar= 50 µm

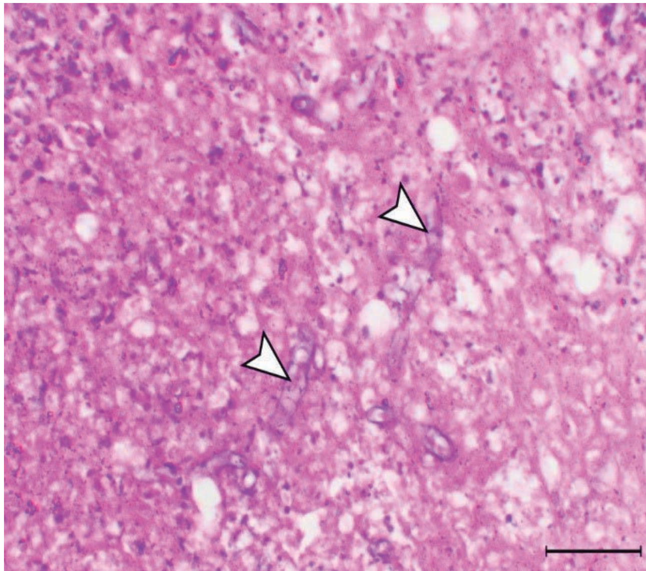

**Figure 3:** The lung of bird showing liquefactive necrosis with presence of basophilic branched hyphae (arrow-heads). H&E, X200, bar= 25 µm

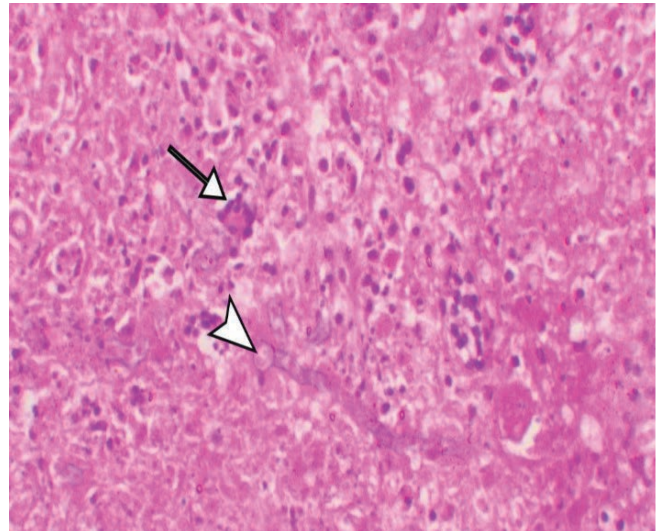

**Figure 4:** The lung of bird showing liquefactive necrosis with presence of basophilic branched hyphae (arrowhead) and infiltration of multinucleated giant cell (arrow), H&E, X200, bar= 25 µm

the drinking water was contaminated with mould, as shown in Table 12.

### *Result of antifungal susceptibility test of moulds isolated from poultry:*

As shown in Table 7, *A. niger* as well as *P. chrysogenum* was resistant to Ketoconazole, while *Cladosporium perangustum* was resistant to Fluconazole. All of the isolated mould was sensitive to Itraconazole, and Nystatin except *A. flavus* that was resistant to Nystatin. All *Aspergillus spp.* were resistant to Fluconazole except *A. niger.*

## Discussion

Fungal infections have their own significance among infectious diseases and appear to be one of the biggest challenges for chicken breeders (15). The combination of bedding material, chicken waste, and litter makes the fungal infection one of the primary poultry infections (16). The present study was conducted aiming to the phenotypic characterization of some fungi isolated from broiler, Baladi, and Saso chicken from different farms in Egypt.

The results of the present study showed that moulds were isolated from the broilers at the highest rate. In addition, the lungs as well as air sacs were the highest organs for mould invasion. These results agreed with a previous report (5, 17) who isolated *A. niger,* and *A. fumigatus* at a high rate from broiler farms. The results of fungal isolation from the studied chicken showed that the highest mould species isolated from the lung, air sacs, liver and trachea were *Aspergillus species* (76.24%, 74.32%, 60%, and 80.32%, respectively), with the most frequent was *A. flavus* compared to *A. fumigatus,* and *A. niger*, while the most frequent fungi isolated from the crop was *Penicillium chrysogenum* (65.9%). These findings were in line with (19) who reported that *Aspergillus spp.*, *Mucor*, and *Penicillium species* were the most prevalent fungal species detected in poultry. In another study that conducted in Egypt, the most prevalent fungal species were *A. niger, C. albicans, A. fumigatus, A. terreus,* and *P. corylophilum* at prevalence rates of 26.6% (45/169), 22.5% (38/169), 20.7% (35/169), 14.8% (25/169), and 15.4% (26/169), respectively (5). The results also were in line with other reports (19, 20) who reported that *Aspergillus species* were the most prevalent fungi, particularly, *A. fumigatus* (2.2%), *A. flavus* (2.6%), *A. niger* (4.4%). Besides, it was reported that the fungal isolates that recovered from bird ceca were *Aspergillus species* (18.4%), followed by *Penicillium* (15.6%), *Verticillium* (6.2%), *Sporidiobolus* (5.2%). In addition, in the ceca, *A. fumigatus* was the most frequent species identified (10.6%), followed by *A. flavus* (5.4%) (21). Aspergillosis, commonly known as brooder's pneumonia, is caused mainly by *Aspergillus fumigatus*, most pathogenic fungi

affecting poultry, but *A. flavus* has also been the culprit associated with many cases (22). It was also reported that the most frequent isolated fungi from chickens were *A. fumigatus* (21.7%) followed by *A. flavus* (19.4%), and *A. niger* (17.1%) (17). In another report (23), it was revealed that *A. fumigatus* was recovered at 28.0%, followed by *A. niger* at 18.6%, and *Penicillium species* at 8.5%.

The present study also revealed that the fungal isolation was the highest in ration (50%), followed by the bedding (28.5%). Unlikely, it was found that chicks' litter and fecal droppings had the highest rate of fungal isolation, followed by drinkers, feeders, air, as well as feeds (5). It was found that the litter (bedding materials) is one of the most contributive factors to fungal contamination in poultry farms (24). In addition, *Aspergillus species*, especially *A. flavus* was the most frequent fungal species isolated from bedding material, water, and air samples (50%, 66.6%, and 50% respectively), followed by *Penicillium chrysogenum* which was recovered from ration, bedding material, and air samples (28.57%, 25%, and 50%, respectively). However, *A. niger* was found at the highest rate in chick droppings, followed by feeds, feeder swabs, and chick litter (5). Meanwhile, the rate found in air samples and drinkers' swabs was higher compared to the rate found in tap water and attendants' hand swabs, which was the lowest. The dispersion of *A. fumigatus* in air samples and feeds, oppositely, was discovered at the highest rate followed by chicks dropping and their litter. It was also reported that the recovered moulds from broiler chicks' surroundings were 6.4% for *A. fumigatus*, 33.3% for *A. flavus*, 2.1% for each of *A. niger*, *A. nidulans* and *A. terrus* as well as 23% for Zygomycetes (19). Likewise, It was stated that there were some of the fungal isolates more frequently identified in the study (24) including *Penicillium* and *Aspergillus species* in fresh, new, and aged litter. In addition, *Cladosporium species* was also frequently isolated in the new litter. Furthermore, air was assessed from poultry habitats for mould contamination (25). The genera *Penicillium species*, *Aspergillus species* were also found in the litter. The existence of fungi in chicken environments indicates poor management approaches, which is the primary source of mould infection of broilers, either in a direct way by fungi or by an indirect way by the fungal mycotoxins. Humidity and temperature conditions seen in chicken farms encourage hyphal growth and effective asexual multiplication, resulting in a large number of easily airborne hydrophobic conidia that are disseminated and inhaled by the birds (26). Increased fungal infections in chicks' environments could be due to increased contamination of the environment with organic waste and sewage, demonstrating poor hygienic procedures and poor ventilation inside the poultry buildings (27).

The histopathological examination of lung nodules of the affected birds revealed central massive liquefactive necrosis involving wide area of the alveolar and bronchial tissues with presence of hyphal elements, with characteristic basophilic septated hyphae, with about 45 angle degree of branched manner, which might be consistent with aspergillosis, and this agrees with previous reports (22, 28, 29, 30).

The present study also showed that the total mould count in the poultry feed samples was higher than that of drinking water samples, where the total mould count in feed samples ranged from 6×cfu/g to 12cfu/g while it ranged from 12 cfu/100 ml to 118 cfu/100 ml in drinking water. These findings were in agreement with a previous study (31), who found that the total mould count (log 10 cfu/g) in poultry feed samples ranged from 1.8 to 4.4 (log 10 cfu/g) while in drinking water samples, it was ranged from 1 to 1.8 (log 10 cfu/ml). Also, it was revealed that the majority of feed samples analyzed contained 1-9 × $10^4$ cfu/g (32).

The antifungal susceptibility testing of moulds isolated from poultry was investigated for the *in vitro* efficacy of antifungals against the representative fungal isolates. The isolated fungal species revealed variable degrees of resistance to the antifungal drugs used in the present study. It was shown that *A. niger* as well as *P. chrysogenum* was resistant to Ketoconazole, while *Cladosporium perangustum* was resistant to Fluconazole. In addition, all the isolated moulds were sensitive to Itraconazole and Nystatin except *A. flavu*s that was resistant to Nystatin. All *Aspergillus spp.* were resistant to Fluconazole except *A. niger.* These results were in agreement with (5) where the susceptibility of all fungal isolates to the medication Itraconazole was determined (100 %), while *Aspergillus spp.* (*A. niger, A. fumigatus, and A. terreus*) showed no susceptibility to fluconazole. In contrast to *A. niger*, which was highly resistant to Nystatin (100%), the other fungal species were very sensitive. Also, it was reported that

*Aspergillus species* were resistant to fluconazole but were sensitive to Nystatin and Voriconazole (33). Unlike the findings of the present study, it was reported that generally all isolates were resistant to fluconazole and Nystatin (4). Highly active triazoles except fluconazole were recommended as the first-line therapy to treat aspergillosis, but their effectiveness is challenged by the emergence of drug resistance (34, 35). The variable degrees of the observed resistance by the *Aspergillus species* could be attributed to the concentration of the antifungal drug applied. It is possible that higher concentrations or higher doses of the drug may be required for effective antifungal effects. It is also likely that some of the strains of the *Aspergillus species* tested in this investigation have developed resistance to the tested antimicrobials.

## Conclusion

It is concluded that *Aspergillus species* is associated with poultry farms in Egypt. Fungi was mainly isolated from ration followed by drinking water. Itraconazole and Nystatin could be applied as proper antifungals for *Aspergillus* infection except for *A. flavu*s that was resistant to Nystatin.

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