Molecular characterization of some fungi isolated from broiler chicken farms

[1]Ashraf, A. Abd El Tawab, [2]Ahmed, A. A. Maarouf, [1]Fatma. I. El-Hofy and [3] Khalid, S.M. Ahmed

[1]Bacteriology, Immunology and Mycology Dep., Fac. Vet. Med. Benha Univ.[2]Animal Health Research "Benhabranch".[3]Animal Health Research "Mansoura branch".

ABSTRACT

The study was performed on 225 chicken (72 diseased and 153 freshly dead ones) from different 45 commercial broiler farms (1- 30 days old) at Dakahlia Governorate for mycotic infection. Samples were taken from these chickens (lung; air sac; crop; liver and brain from each chicken) after clinical and postmortem examination for mycological examination. The results revealed that, fungi were isolated from 503 positive samples (44.7%); represented as 119 positive samples (10.6%) from diseased chickens and 384 positive samples (34.1 %) from freshly dead ones.651 fungal isolates (moulds and yeasts) were isolated from 1125 samples, where 219(33.6%) were isolated from lung samples followed by 199(30.6%); 119(18.3%); 91(14.0%) and 23(3.5%) from air sac, liver, crop and brain samples respectively. Moreover, *A. fumigatus* was the most isolated one 141(21.7%) followed by *A. flavus* 126(19.4%); A. niger 111(17.1%); *Rhizopus spp.* 67(10.3%); *C. albicans* 58(8.9%); Mucor spp. 42(6.5%); *Penicillium spp*. 32(4.9%); *A. terreus* 20(3.1%); Fusarium spp.19 (2.9%); C. krusei 13(2.0%); C. tropicalis 9(1.4%); *A. candidus* 9(1.4%) and finally A. *ochraceus* 4(0.6%). PCR using fungus-specific universal primer pairs (ITS1 and ITS4) was used for identification and genotypic characters of Aspergillus species *(A. fumigatus; A. flavus and A. niger*).They were able to successfully amplify the ITS1–5.8S rDNA region of all tested Aspergillus isolates, providing a single PCR product of about 600 bp for all tested isolates PCR using fungus-specific universal primer pairs (ITS and RPS) was used for identification and genotypic characters of C. providing a single PCR product of about 109 bp .Sequence of the internal transcribed spacer 1 reigon (ITS-1) of isolated A. flavus Gene Bank accession number for studied nucleotide sequence (Bankit 1867303) is KM 983253. The sequences obtained for ITS-1 region were more than 98% identical to the corresponding GenBank sequences (accession no. DQ467968.1; KC994648.1; LN482516.1 and KP689246.1). Sequence of the internal transcribed spacer 1 region (ITS-1) of isolated *C. albicans*. Gene Bank accession number for studied nucleotide sequence (Bankit 1867955) is KM 983254. The sequences obtained for ITS-1 region obtained were more than 95% identical to the corresponding GenBank (accession no. M87288.1; gb|S71769.1|S71769; emb (AL033396.1) and AP006852.1).

Key wards: *C. albicans, A. flavus,* A. *ochraceus*

(http://www.bvmj.bu.edu.eg) **(BVMJ-29(2): 106-118, 2015)**

1. INTRODUCTION

Fungal infections are common in all kinds of poultry birds but are less prevalent as compared to bacterial and viral infections. Fungi are eukaryotic organisms, comprising of both yeasts and molds. They cause significant economic losses to the poultry industry either due to their direct infectious nature or due to production of mycotoxins resulting in high morbidity and mortality, especially in young birds and cause stunted growth; diarrhea; and fatal encephalitis (Singh et al., 2012). Risk factors, which predisposes to fungal infection and aggravate disease include malnutrition, vitamin D deficiency, poor hygiene, prolonged use of antibiotic suppressing normal bacterial flora, stress an immunosuppressive disease (Velasko,

2000). Aspergillosis and Candidiasis are the most important fungal diseases of poultry (Dhama et al., 2013a). Aspergillosis is a necrotizing and granulomatous cavity disease of the lungs with hematogenous spread caused mainly by Aspergillus fumigatus, most pathogenic fungi affecting poultry (Redig, 2005; Ganguly et al., 2011 and Dhama et al., 2013b). *A. fumigatus* infection occurs more frequently in poultry, as the spores of this pathogen species are smaller than those of other Aspergillus spp. (Dhama et al., 2013b). Other Aspergillus spp. that may affect birds adversely are *A. flavus, A. terreus, A. glaucus, A. nidulans* and *A. niger* (Beemaert et al., 2010and Dhama et al., 2013b). Affected birds may show gasping, dyspnea, fever, foetid yellowish diarrhea, rapid loss of condition with convulsions together with anemia and mortality range between 5-50%. Survivors often develop chronic disease due to pulmonary insufficiency or neurological fungal metastasis and may become lethargic and stunted (Atasever and Gumussoy, 2004; Beemaert et al., 2010 and Dhama et al., 2013b). Candidiasis is a fungal disease caused by yeasts of the genus Candida having nearly 200 species, among them, six are most frequently isolated. While C. albicans is the most abundant and significant species, *C. tropicalis, C. glabrata, C. parapsilosis, C. krusei* and *C. lusitaniae* have also been implicated as causative agents (Tiwari et al., 2011). C. albicans is an asporogenous and pseudomycelial dimorphic yeast having fermentation capability. It grows on ordinary media over a wide range of pH and temperature. Budding yeast forms (blastospores) are 3-4 um on epithelial surfaces whereas branching septate hyphae or pseudohyphae are 3-5 um diameter in deeper tissues. It can utilize ammonia but not nitrate; nitrogen and most strains need growth factor biotin to be supplemented for their growth (Novak et al., 2003). The identification of fungi by traditional microscopic, cultural and metabolic characteristics remains commonly used. The species level identification is very complex. Macro-morphological identification is done based on conidial and mycelial color, colony diameter, colony reverse color, production of exudates and soluble pigments. Microscopic identification is mainly dependent on seriation, shape and size of vesicle, conidia and stipe morphology (Chakranarayan and Pati, 2013). These methods are laborious, time-consuming (may take days to weeks), require significant technological expertise and sometimes unsuccessful because of the atypical features of some isolates. Therefore, Molecular approaches have been developed to provide more rapid and accurate identification of pathogenic fungi comparing to traditional phenotypic methods (Makimura et al., 1994 and Yamakami et al., 1996). The internal transcribed spaces 1 and 2 regions (ITS 1 and ITS 2) have been used extensively for molecular analysis of fungi. length

2. MATERIAL AND METHODS

2.1. Samples collection

A total of 225 chicken were collected from different 45 commercial broiler farms (1- 30 days old) at Dakahlia Governorate were inspected for mycotic infection. Samples were taken from 72 diseased chicken and 153 freshly dead ones (lung; air sac; crop; liver and brain from each chicken) after clinical and postmortem examination. Each examined organ was taken alone in sterile plastic bags, kept in icebox and transferred with minimum delay to the laboratory for mycological examination.

2.2. Mycological culture examination

The surface of organs were seared by hot spatula, then a sterilized loopful was introduced through seared portion of the organ. Loopfuls were inoculated on modified Sabouraud's dextrose broth under aseptic condition and incubated aerobically at 37° c for 3-days. Two Loopfuls were taken, the first was streaked on the following media: Sabouraud's dextrose

agar media; Malt Extract agar and Rose Bengal agar and incubated for 10 days at 37° c. and fungal growth colonies were picked up and kept in Sabouraud dextrose agar slopes and Malt Extract slope ager then incubated at 22-25°C for 5-7 days. The purified colonies were identified by careful observation and measurements of the macroscopically and microscopically characteristics of the mould colonies according to Jand and Singh (1995); David et al., (2007) and Markey et al., (2013). The second was streaked on Sabouraud's dextrose agar media and incubated for 48h at 30°C. The yeast growth colonies were sub- cultured onto the following media: Eosin methylene blue agar; Rose Bengal agar and CHROM Candida agar and incubated at 30°C for 48 h. The yeast growth colonies were picked up and kept in Sabouraud dextrose agar slopes then incubated at 22-25°C for 5-7 days. The purified colonies were identified by careful observation and measurements of the macroscopically; microscopically characteristics of the yeast colonies and biochemically according to Lodder and Krieger Van Rij (1970); David et al., (2007) and Markey et al., (2013).

2.3. Genotypic methods of identification:

PCR was applied by using fungus-specific universal primer pairs (ITS1 and ITS4) was used for identification and genotypic characters of15 random isolated Aspergillus species (*A. fumigatus; A. flavus and A. niger*) and (ITS and RPS) for 5 random isolated *C. albicans*, following QIAamp DNeasy Plant Mini kit instructions (Catalogue no. M501DP100), Emerald Amp GT PCR master mix (Takara) with Code No. RR310Aand 1,5% agarose gel electrophoreses (Sambrook et al., 1989).

3. RESULTS

3.1. Clinical and postmortem examinations:

The clinical examination of studied chicken showed clinical manifestations as dyspnea, gasping, accelerated breathing, depression, emaciation, ruffled feathers, over distention of crops with watery stink smelling discharge from the mouth, profuse watery diarrhea, blindness, torticollis, lack of equilibrium, and stunting growth. Meanwhile, the postmortem lesions of diseased and freshly dead chicken from which fungi (moulds and yeasts) were isolated showed congestion of the lungs, air saculitis, ulcer formation in the crop with excessive secretion covering the inner surface of the crop, mucous enteritis with sloughing of the intestinal mucosa and some of them showed greenish gray lesions and caseated nodules of 1-2mm thickness distributed in lungs, livers, proventriculus, gizzard intestine and abdomen.

3.2. Isolation and identification of fungi (moulds and yeasts) isolates

Prevalence of positive samples for fungi isolation from examined chickens broiler farms

The results of fungi (moulds and yeasts) isolation (Table1) showed that, 503 out of 1125 samples (44.7%) were positive for fungi isolation, where 119 positive samples (10.6%) from diseased chickens and 384 positive samples (34.1 %) from freshly dead ones. Moreover, the highest positive samples in diseased and freshly dead chickens were in both lung (43&128) and air sac (35&119) samples. Most samples showed mixed fungi (moulds and yeasts).

Total number and percentage of fungi isolated from examined chickens broiler samples. The results of mycological examination (Table, 2) revealed that, a total number of 651 fungal isolates (moulds and yeasts) were isolated from 1125 samples, where 219(33.6%) were isolated from lung samples followed by 199(30.6%); 119(18.3%); 91(14.0%) and 23(3.5%) from air sac, liver, crop and brain samples respectively. Moreover, *A. fumigatus* was the most isolated one 141(21.7%) followed by *A. flavus* 126(19.4%); *A. niger* 111(17.1%); Rhizopus spp. 67(10.3%); *C. albicans* 58(8.9%); Mucor spp. 42(6.5%);

Penicillium spp. 32(4.9%); *A. terreus* 20(3.1%); *Fusarium* spp.19 (2.9%); *C. krusei* 13(2.0%); *C. tropicalis* 9(1.4%); *A. candidus* 9(1.4%) and finally A. *ochraceus* 4(0.6%) 3.2.1.2. Total number and percentage of fungal species isolated from examined organs. Out of 651 fungi isolated from examined organs, 411(63.1%) Aspergillus species were isolated mostly from lung samples 162 (39.4%), followed by 142(34.6%), 64(15.6%), 37(9.0%) and 6(1.5%) from air sacs, liver, crops and brain samples respectively. Other mould species isolated were160 (24.6%) mostly from air sac samples55 (34.4%) followed by 54(33.8%); 22(13.8%); 17(10.6%) and 12(7.5%) from lung, liver, brain and crop samples respectively. Moreover, Candida species isolated were 80 (12.3%) mostly from crop samples 42(52.5%) followed by 33(41.3%); 3 (3.7%) and 2 (2.5%) from liver, lung and air sac samples respectively meanwhile, they could not isolated from brain samples (Table, 2). Total number and percentage of fungal species isolated from lungs; air sacs; crops; liver and brain of studied chickens were tabulated in (Tables 3-7).

3.3. Identification of fungi isolates using polymerase chain reaction (PCR) (Genotypic characters)

Genotypic characters of Aspergillus species (*A. fumigatus; A. flavus and A. niger*)
PCR using fungus-specific universal primer pairs (ITS1 and ITS4) was used for identification and genotypic characters of Aspergillus species (*A. fumigatus; A. flavus and A. niger*). They were able to successfully amplify the ITS1–5.8S rDNA region of all tested Aspergillus isolates, providing a single PCR product of about 600 bp for alltested isolates as shown in photo (1, 2 & 3).
Genotypic characters of C. albicans. PCR using fungus-specific universal primer pairs (ITS and RPS) was used for identification and genotypic characters of C. albicans. They were able to successfully amplify the ITS1–5.8S rDNA region of all tested *C. albicans* isolates, providing a single PCR product of about 109 bp for all tested isolates as shown in fig (4).Sequence of the internal transcribed spacer 1 reigon (ITS-1) of isolated *A.flavus* Gene Bank accession number for studied nucleotide sequence (Bankit 1867303) is KM 983253. The sequences obtained for ITS-1 reigon were more than 98% identical to the corresponding GenBank sequences (accession no. DQ467968.1; KC994648.1; LN482516.1 and KP689246.1).
Sequence of the internal transcribed spacer 1 region (ITS-1) of isolated C.albicans Gene Bank accession number for studied nucleotide sequence (Bankit 1867955) is KM 983254. The sequences obtained for ITS-1 region obtained were more than 95% identical to the corresponding GenBank (accession no. M87288.1; gb|S71769.1|S71769; emb (AL033396.1) and AP006852.1). Finally, Aspergillus species mainly A. fumigatus; A. flavus; A. niger and C. albicans were the most important causes of mould infection and candidiasis in broiler chicken farms.

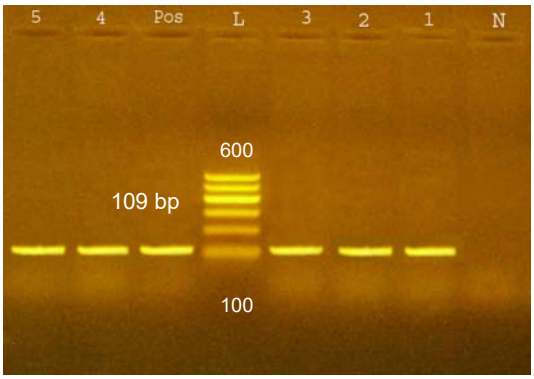

Fig. (1). c. albicans I^{ts1} L.100bp – 600bpDNA ladder, N. negative control, Pos. positive control (at 109bp), Lane 1 to 5 positive C. albicanis

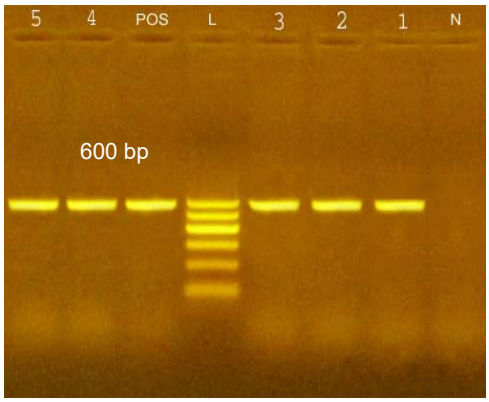

Fig. (2) A. Flavus ITS1L. 100 -600bp DNA Ladder POS. positive control (at 600bp) N. negative control. L1 to 5 positive A.Flavus

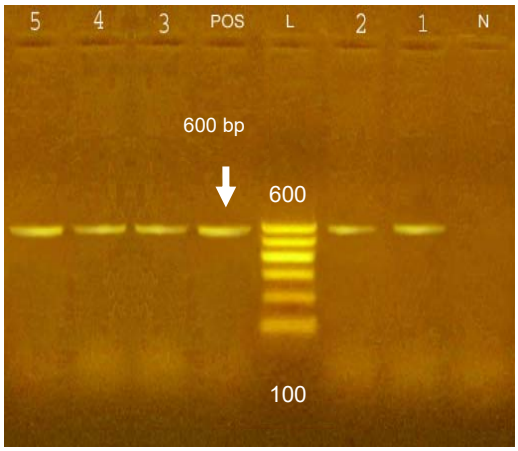

Fig (3). A. Fumigatous ITS1, L. 100-600bp DNA Ladder, P OS. Positive control N. negative control, L1 to 5 positive A. Fumigatous

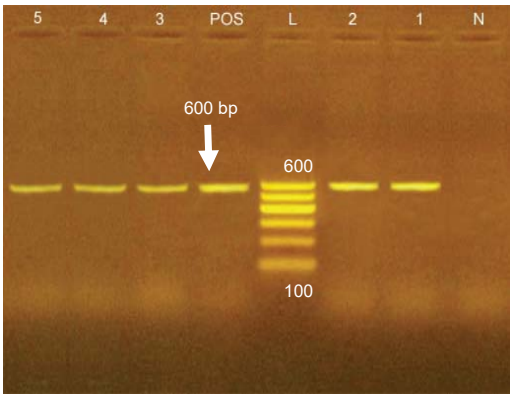

Fig (4) A. Niger I^{TS} L.100-600bp DNA Ladder, POS. positive control at 600bp
N. negative control, L1 to 5 positive A. Niger

Table (1): Total number and Percentage of positive samples for fungi isolation from studied chickens

Chicken case	Number of sample	Number of positive samples						Positive percentage		
		Lung	Air sacs	Crop	Liver	Brain	Total	%[1]	%[2]	%[3]
Diseased (72)	360	43	35	17	19	5	119	33.1	23.7	10.6
Freshly dead (153)	765	128	119	54	70	13	384	50.2	76.3	34.1
TOTAL(225)	1125	171	154	71	89	18	503	44.7	100.0	44.7

[1]Percentage in relation to total number of cases in each row. [2] Percentage in relation to total number of positive samples (503). [3] Percentage in relation to total number of collected samples (1125)

4. DISCUSSION

The mycotic infection of chicken is one of the most serious problems that affect chicken causing high economic losses due to not only high morbidity and mortality in young chicken but also they are the leading cause of immunosuppression in birds, lowering their resistance level to various viral and bacterial diseases and increased mortality. On the other hand, an increase in the incidence of the mycotic diseases can be expected due to the wide use of antibiotics preparations in the treatment of many diseases as well as the extensive use of antibiotic as feed additives, which enhance mycotic complications. Therefore, this study was planned for mycological examination in broiler chicken farms. The results of clinical and postmortem examinations of studied chicken were similar to that reported by (Sajid et al.,2006 ; Zafra et al., 2008; Cacciuttolo et al.,2009; Arne et al.,2011; Dhama et al.,2011;Dhama et al.,2013 ; Amin et al., 2014 ; Musa et al.,2014 and Salem and Ali , 2014). The results of fungi isolation, (Table, 1) revealed that 503 samples (44.7%) out of 1560 samples were positive for fungi isolation; represented as 119 positive samples (10.6%) from diseased chickens and 384 positive samples (34.1 %) from freshly dead ones. Moreover, the highest positive samples in diseased and freshly dead chickens were in both lung (43&128) and air sac (35&119) samples. These results came in accordance with that obtained by (Zhao et al., 1994; Garcia et al., 2003; Steinlage et al., 2003 and Sajid et al., 2006). Culturing is regarded as the gold standard for confirming diagnosis of fungal infection with variable sensitivity due to sample collection techniques, storage and transport conditions (Bradsher et al., 2003 and Koneman et al., 2007). The use of different media for fungi isolation was performed to avoid false results and ensure accuracy. This is because misidentification of fungi species has been previously reported in results from the use of single identification media (Lo et al., 2001). Also, they helps to accelerate growth rate and the production of conidia (Odds and Bernaerts, 1994; Baumgartner et al., 1996; Chakranarayan and Pati, 2013). Moreover, Culture CHROM agar contains a chromogenic, 5-bromo-6-chloro -3- indolyl phosphate P-toluidine (chromogenic substrates) and 5-bromo-4-chloro-3-indolyl/ N-acetyl-P-D-glucosaminide, which reacts with species specific enzymes to give yeasts colonies which are different in colour (Ghelardi et al., 2008).

Table (2): Incidence of fungi (moulds and yeasts) isolated from different organs of studied chickens in relation to number of isolates

Fungus	Lung			Air Sac			Crop			Liver			Brain			Total	
	No	%*	%**	No	%*	%**	No	%*	%**	No	%*	%**	No	%*	%**	No	%***
Aspergillus spp.																	
A.fumigatus	55	25.1	39.0	57	28.6	40.4	8	8.8	5.7	15	12.6	10.6	6	26.1	4.3	141	21.7
A.flavus	48	21.9	38.1	45	22.6	35.7	7	7.7	5.6	26	21.9	20.6	0	0.0	0.0	126	19.4
A.niger	39	17.8	35.1	32	16.1	28.8	17	18.7	15.3	23	19.3	20.7	0	0.0	0.0	111	17.1
A.candidus	6	2.7	66.7	3	1.5	33.3	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	9	1.4
A.terreus	11	5.0	55.0	4	2.0	20.0	5	5.5	25.0	0	0.0	0.0	0	0.0	0.0	20	3.1
A.ochraceus	3	1.4	75.0	1	0.5	25.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	4	0.6
Total Aspergillus spp.	162	74.0	39.4	142	71.3	34.5	37	40.7	9.0	64	53.8	15.6	6	26.1	1.5	411	63.1
Other fungal species																	
Rhizopus spp.	21	9.6	31.3	20	10.1	29.9	4	4.4	6.0	5	4.2	7.5	17	73.9	25.4	67	10.3
Mucor spp.	16	7.3	38.1	18	9.1	42.9	3	3.3	7.1	5	4.2	11.9	0	0.0	0.0	42	6.5
Fusarium spp.	6	2.7	31.6	6	3.0	31.6	2	2.2	10.5	5	4.2	26.3	0	0.0	0.0	19	2.9
Penicillium spp.	11	5.0	34.4	11	5.5	34.4	3	3.3	9.4	7	5.9	21.9	0	0.0	0.0	32	4.9
Total other spp.	54	24.6	33.8	55	27.6	34.4	12	13.2	7.5	22	18.5	13.7	17	73.9	10.6	160	24.6
Candida spp.																	
C. albicans	3	1.4	5.2	2	1.0	3.4	40	44.0	69.0	13	10.9	22.4	0	0.0	0.0	58	8.9
C. krusei	0	0.00	0.00	0	0.0	0.0	2	2.2	15.4	11	9.2	84.6	0	0.0	0.0	13	2.0
C. tropicalis	0	0.00	0.00	0	0.0	0.0	0	0.0	0.0	9	7.6	100.0	0	0.0	0.0	9	1.4
Total Candida spp.	3	1.4	3.7	2	1.0	2.5	42	46.2	52.5	33	27.7	41.3	0	0.0	0.0	80	12.3
Grand total	219	100.0	33.6	199	100.0	30.6	91	100.0	14.0	119	100.0	18.3	23	100.0	3.5	651	100.0

* Percentage in relation to total number of isolates in each column,

** Percentage in relation to total number of isolates in each row. ***Percentage in relation to total number of isolates (651)

Table (3): Total number and percentage of fungi (moulds and yeasts) isolated from lung samples of studied chickens

Fungus	NO.	%*	%**
A. Fumigatus	55	25.1	24.4
A. flavus	48	21.9	21.3
A. niger	39	17.8	17.3
A. candidus	6	2.7	2.7
A. terreus	11	5.0	4.9
A. ochraceus	3	1.4	1.3
Total Aspergillus spp.	162	74.0	72.0
Rhizopus spp.	21	9.6	9.3
Mucor spp.	16	7.3	7.1
Fusarium spp.	6	2.7	3.1
Penicillium spp.	11	5.0	4.9
Total other mould spp.	54	24.6	24.0
C. albicans	3	1.4	1.3
C. krusei	0	0.00	0.0
C. tropicalis	0	0.00	0.0
Total Candida spp.	3	1.4	1.3
Grand total	219	100.0	97.3

* Percentage in relation to total number of isolates (219). ** Percentage in relation to total number of examined lung samples (225).

Table (4): Total number and percentage of fungi (moulds and yeasts) isolated from air sacs samples of studied chickens

Fungus	NO.	%*	%**
A.fumigatus	57	28.6	25.3
A.flavus	45	22.6	20.0
A.niger	32	16.1	14.2
A.candidus	3	1.5	1.3
A.terreus	4	2.0	1.8
A. ochraceus	1	0.5	0.4
Total Aspergillus spp.	142	71.3	63.1
Rhizopus spp.	20	10.1	8.9
Mucor spp.	18	9.1	8.0
Fusarium spp.	6	3.0	2.7
Penicillium spp.	11	5.5	4.9
Total other mould spp.	55	27.6	24.4
C. albicans	2	1.0	0.9
C. krusei	0	0.0	0.0
C. tropicalis	0	0.0	0.0
Total Candida spp.	2	1.0	0.9
Grand total	199	100.0	88.4

* Percentage in relation to total number of isolates (199). ** Percentage in relation to total number of examined air sacs samples (225).

Table (5): Total number and percentage of fungi (moulds and yeasts) isolated from crop samples of studied chickens

Fungus	NO.	%*	%**
A.fumigatus	8	8.8	3.6
A.flavus	7	7.7	3.1
A.niger	17	18.7	7.6
A. candidus	0	0.0	0.0
A.terreus	5	5.5	2.2
A. ochraceus	0	0.0	0.0
Total Aspergillus spp.	37	40.7	16.4
Rhizopus spp.	4	4.4	9.3
Mucor spp.	3	3.3	1.3
Fusarium spp.	2	2.2	0.9
Penicillium spp.	3	3.3	1.3
Total other mould spp.	12	13.2	5.3
C. albicans	40	44.0	17.8
C. krusei	2	2.2	0.9
C. tropicalis	0	0.0	0.0
Total Candida spp.	42	46.2	18.7
Grand total	91	100.0	40.4

* Percentage in relation to total number of isolates (91). ** Percentage in relation to total number of examined crop samples (225).

Table (6): Total number and percentage of fungi (moulds and yeasts) isolated from liver samples of studied chickens

Fungus	NO.	%*	%**
A. fumigatus	15	12.6	6.7
A. flavus	26	21.9	11.6
A. niger	23	19.3	10.2
A. candidus	0	0.0	0.0
A. terreus	0	0.0	0.0
A. ochraceus	0	0.0	0.0
Total Aspergillus spp.	64	53.8	28.4
Rhizopus spp.	5	4.2	2.2
Mucor spp.	5	4.2	2.2
Fusarium spp.	5	4.2	2.2
Penicillium spp.	7	5.9	3.1
Total other mould spp.	22	18.5	9.8
C. albicans	13	10.9	5.8
C. krusei	11	9.2	4.9
C. tropicalis	9	7.6	4.0
Total Candida spp.	33	27.7	14.7
Grand total	119	100.0	52.9

* Percentage in relation to total number of isolates (119). ** Percentage in relation to total number of examined liver samples (225).

Table (7): Total number and percentage of fungi (moulds and yeasts) isolated from brain samples of studied chickens

Fungus	NO.	%*	%**
A. fumigatus	6	26.1	2.7
A. flavus	0	0.0	0.0
A. niger	0	0.0	0.0
A. candidus	0	0.0	0.0
A. terreus	0	0.0	0.0
A. ochraceus	0	0.0	0.0
Total Aspergillus spp.	6	26.1	2.7
Rhizopus spp.	17	73.9	7.6
Mucor spp.	0	0.0	0.0
Fusarium spp.	0	0.0	0.0
Penicillium spp.	0	0.0	0.0
Total other mould spp.	17	73.9	7.6
C. albicans	0	0.0	0.0
C. krusei	0	0.0	0.0
C. tropicalis	0	0.0	0.0
Total Candida spp.	0	0.0	0.0
Grand total	23	100.0	10.2

* Percentage in relation to total number of isolates (23). ** Percentage in relation to total number of examined brain samples (225).

The main features of isolated fungi in the present study is concided with the description of Moneer (2008); Tartor (2010); Taha (2011); Klich (2002); Mohamed (2012); Chakranarayan and Pati (2013) and Musa et al., (2014). Rapid and accurate differentiation of pathogenic species of Aspergillus and Candida has become particularly important for selecting effective antifungal therapy. In addition, information about species identity is important for epidemiological and control purposes, such as for outbreaks of invasive aspergillosis, for surveillance of the emergence of new species and accurate determination of incidence rates (Henryet al., 2000; Hinrikson et al., 2005). Moreover, the molecular characterization of Aspergillus spp. and C. albicans carried out by using of PCR and sequencing of the PCR products. The identification systems for Aspergillus spp. and C. albicans depend on the PCR were based on using 18S or 28S rDNA as target DNA. However, the sequences in these regions are conserved across a wide range of fungi; it is therefore difficult to be used for identification of Aspergillus spp. The ITS region contains variable elements that allow sequence-based identification of Aspergillus species and C. albicans; therefore, the region offers a possible template for identification of different Aspergillus spp. and C. albicans either by using primers designed from this region for the different spp. or sequencing of the amplified region by using primers for amplification of the ITS region (Lim and Lee, 2000 and Makimura, 2001). Regarding to Aspergillus species, in this study, the primers used for identification of PCR designed for amplification of the ITS region (Mirhendi et al., 2007). The examined 15 samples were identified as A.fumigatus, A.flavus and A.niger by the traditional methods and all 15 samples were successfully amplified a product of 600 bp and give + ve with the PCR examination which confirm the results of the traditional methods. The PCR products of 15 samples which were identified as A. fumigatus, A. flavus and A. niger were subjected for sequencing. These results came in accordance with those recorded by Sujita et al., (2004); Mirhendi et al., (2007) and

Khaphagy et al., (2012). Regarding to C. albicans, in this study, the primers used for identification of PCR designed for amplification of the ITS region (Tarini et al.,2010). The examined 5 samples were identified as C. albicans by the traditional methods and all 5 samples were successfully amplified a product of 109 bp and give + ve with the PCR examination which confirm the results of the traditional methods. The PCR products of 5 samples which were identified as *C. albicans* were subjected for sequencing. These results came in accordance with those recorded by Tarini et al., (2010).

5. REFERENCES

A1- Dahshan, E.M.E .2003. Mycological studies on Respiratory Infection in Poultry. Ph. Thesis (Bacteriology, Immunology and Mycology) Fac. Vet. Med. Cairo University

Amin, H.; Zali, M.H.S.; Seyedgholizadeh, S.; Malekan, M, Yahyapour, H. 2014. Prevalence of subclinical Candidiasis in broiler flocks Urmia, Iran. Scholars Journal of Agriculture and Veterinary Sciences, 1(4):153-155.

Arne, P.; Thierry, S.; Wang, D.; Deville, M. and Le Loch, G.2011. Aspergillus fumigatus in poultry. Int. J. Microbiol., 10:1155-1169.

Atasever, A. and Gumussoy, K.S. 2009. Pathological, clinical and mycological findings in experimental aspergillosis infections of starlings. J. Vet. Med. A, Physiol. Pathol. Clin. Med., 51: 19-22.

Baumgartner, C.; Freydiere, A. and Gille, Y.1996. Direct Identification and Recognition of Yeast Species from Clinical Material by Using Albicans ID and CHROM agar Candida Plates. J. Of Clinical Microbiology, 34 (2): 454–456.

Beemaert, L.A.; Pasmans, F.; Waeyenberghe, L.V.; Haesebrouck, and Martel, A. 2010. Aspergillosis infection in birds: A review. Avian Pathol., 39: 325-331.

Bradsher, R.W.; Chapman, S.W. and Pappas P.G. 2003. Blastomycosis. Infectious Diseases Clinical North America, 17:21-40.

Cacciuttolo, E.; Rossi, G.; Nardoni, S.; Legrottaglie, R. and Mani, P. 2009. Anatomo-pathological aspects of avian aspergillosis. Vet. Res. Commun., 33: 521-527.

Chakranarayan, M.and Pati, A. 2013. Comparison of microscopic, macro morphological and aflatoxin producing capabilities of Aspergillus species associated with rhizosphere of groundnut (A. hypogaea L.). J. Chem. Bio. Phy. Sci., 3(2):1327-1337.

Daniele, C.; Arianna, T.; Federica, G.; Crocifissa, S.M.; Ilaria, B.;Fabio, B.; Roberto B.; Stefano, L.; and Sonia S. 2008.DNA microarray based on arrayed-primer extension technique for identification of pathogenic fungi responsible for invasive and superficial mycoses. J. of Clinical Microbiology, 46 (3): 909–915.

David, E.; Davis, S.; Alexiou; H.; Handke, R. and Bartley, R. 2007. Descriptions of Medical Fungi. Second Ed. Bibliography.

Dhama, K.; Chakraborty, S.; Wani, Mahima, M.Y. and Verma, A.K. 2013a. Novel and emerging therapies safeguarding health of humans and their companion animals: A review. Pak. J. Biol. Sci., 16: 101-111.

Dhama, K.; Tiwari, R. and Basaraddi, M.S. 2011. Avian diseases transmissible to humans. Poult. Technol., 6: 28-32.

Dhama, K.; Chakraborty, S.; Kumar A. V.; Tiwari, R.; Barathidasan, R. and Singh, S.D.2013b. Fungal/Mycotic Diseases of Poultry-diagnosis, Treatment and Control: A Review. Pakistan Journal of Biological Sciences 16 (23): 1626-1640.

Dhamaakraborty, S.; Verma, K.; Ch A.K.; Tiwari, R.; Barathidasan, R.; Kumar, A. and Singh, S. D.2013. Fungal/Mycotic Diseases of Poultry-diagnosis, Treatment and Control: A Review. Pakistan J. Biological Sciences, 16(23):1626-1640.

El- Bassiouni, A.A.; El- Ged, A.S.; Edris, A. and A.M. Khalid, A.M. 1988. Aspergillus species isolated from chickens and related human workers. Alex. J. vet. Sci., 4(2):205-211.

Ganguly, S.; Paul,I.and Mukhopadhayay, S.2011. Systemic impact of Aspergillosis or brooder's pneumonia in birds. Poult. Line, 11: 49-49.

Ghelardi, E.; Pichierri, G.; Castagna, B.; Barnini, S.; Tavanti, A. and Campa, M. 2008. Efficacy of Chromogenic Candida Agar for isolation and presumptive identification of pathogenic yeast species. Clinical Microbiology Infectious, 14: 141-147.

Henry, T.; Iwen, P.C. and Hinrichs, S. H. 2000. Identification of Aspergillus species using internal transcribed spacer regions 1 and 2. J. Clin. Microbiol.,38: 1510–1515.

Hinrikson, H. P.; Hurst, S. F.; Lott, T. J.; Warnock, D. W. and Morrison, C. J. 2005. Assessment of ribosomal large-subunit D1–D2, internal transcribed spacer 1, and internal transcribed spacer 2 regions as targets for molecular identification of medically important Aspergillus species. J. Clin. Microbiol., 43: 2092–2103.

Khaphagy, A.A.; Enany, M. E1. and Nada, H. E.2012. Polymerase chain reaction amplification of the ITS-1 region and sequencing of the amplified products of most pathogenic Aspergillus spp. (A. fumigatus, A. flavus & A. niger). SCVMJ, XVII (I):1-8.

Klich, M.A.2002. Identification of common Aspergillus species. CBS Ultrecht, Nether Lands.

Koneman , E.; Ellen, S. and Janda,W. 2007. Atlas and textbook of diagnostic microbiology. Philadelphia: Lippincott-Raven.

Lim, Y.H. and Lee, D.H. 2000. Rapid PCR Method for detecting *Candida albicans* Using Primers derived from the Integrin-like Protein Gene αINT1of *Candida albicans.* The Journal of Microbiology, 38 (2):105-108.

Lo, H.J.; Ho, Y.A. and Ho, M., 2001: Factors accounting for misidentification of Candida species. Journal Microbiology Immunology Infections, 34:171-177.

Lorin, D. 2013. Evaluation of fungal incidence in broiler farm. Scientific works series C. veterinary medicine, vol. LIX (1):105-109.

Makimura, K.2001. Species identification system for dermatophytes based on the DNA sequences of nuclear ribosomal internal transcribed spacer 1. Nippon Ishinkin Gakkai Zasshi, 42: 61- 67.

Makimura, K.; Murayama, S.Y. and Yamaguchi, H. 1994. Specific detection of Aspergillus and Penicillium species from respiratory specimens by polymerase chain reaction (PCR). Japan J. Med. Sci. Biol., 47:141 - 156.

Markey, B.K.; Leonard, F.C.; Archambault, M.; Cullinane, A. and Maguire, D. 2013. Clinical Veterinary Microbiology. Second edition. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.

Mirhendi, H.; Diba, K.; Kordbacheh, P.; Jalalizand, N. and Makimura, K.2007. Identification of pathogenic Aspergillus species by a PCR restriction enzyme method. J. Medical Microbiology,56: 1568-1570.

Moneer, E.M.El. S. 2008. studies on candida albicans isolated from healthy and diseased birds. Ph.D. Thesis, (Bacteriology, Immunology and Mycology) Fac. Vet. Med., Zagazig University.

Musa, I.W.; Aliyu, G. and Ismail, A.2014. Aspergillosis in Broilers: Reports of three cases from a commercial and two Broiler Breeder farms in Zaria, Nigeria. Int. J. Curr. Microbiol. App. Sci ., 3(6): 932-938.

Novak, A.; Vagvolgyi, C .and Pesti, M. 2003. Characterization of Candida albicans colony morphology mutants and their hybrids. Foia Microbiol., 48: 203-209.

Odds, F. C. and Bernaerts, R. 1994. CHROM agar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. J. Cin. Microbiol. 32 (8): 1923 – 1929.

Redig, P. 2005. Mycotic infections in birds I: Aspergillosis. Proceedings of the North American Veterinary Conference, January 8-12, 2005, Eastern States Veterinary Association, Orlando, FL., USA, pp.: 1192-1194.

Refai, M.K.; Attia, S.A.; Salem, R.M. and Al-Dahshan, E.M.2004. Studies on the pathogenicity and enzymatic activities of Aspergillus fumigatus, A. Flavus and A. niger isolated from chickens and their environment, Egypt. J. comp. Path. & clinic. Path. 17(2):193- 205.

Sajid, M.A.; Khan, I.A. and Rauf, U. 2006. Aspergillus fumigatus in commercial flocks, a serious threat to poultry

industry in Pakistan. J.Anim.Sci.,16(3-4):15-18.

Salem, Lobna, M. A. and Ali, A. 2014. Epidemiological study of Aspergillosis in chickens and human contacts in chicken farms at Kalyoubia Governorate. J.Agriculture and Veterinary Science (IOSR-JAVS), 7(7):20-24

Sambrook, J.; Fritscgh, E.F. and Mentiates 1989. Molecular coloning. A laboratory manual. Vol., Cold spring Harbor Laboratotry press, New York.

Spanamberg, G.A.; Renata, G.; Gabriela, Miller, S.and Cibele, Floriano, F.2013. Aspergillus fumigatus from normal and condemned carcasses with air Sacculitis in commercial poultry. Pesqu. Vet. Bras., 33(9):1071 -1075.

Sujita, C.; Makimura, K.; Uchida, K.; Yamaguchi, H. and Nagai, A. 2004. PCR identification system for the genus Aspergillus and three major pathogenic species: A. fumigatus, A. flavus and A.niger. Med. Mycol.J., 42 :433-437.

Taha, M. C.2011. Medical Mycology: Atlas of Medically important fungi and dermatomycosis.1st. Ed. Mecca Printing House Cairo Egypt.

Tarini, N.M.A.; Wahid, M.H.; Ibrahim, F.; Yasmon, A. and Djauzi, S. 2010. Development of multiplex-PCR assay for rapid detection of Candida spp. Med. J. Indones., 19(2) May 2010.

Tartor, Y.H. I. 2010. Studies on methods used for identification of yeasts isolated from human and animals. M.V. Sc. Thesis, Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Zagazig University.

Tiwari, R.; Wani ,M.Y.and Dhama, K. 2011. Candidiasis (moniliasis, thrush or sour crop) in poultry: An overview. Poult. Technol., 6: 110-111.

Velasko, M.C. 2000. Candidiasis and Cryptococcosis in birds. Semin. Avian Exotic Pet Med., 9: 75-81.

Yamakami, Y.; Hashimoto, A .; Tokimatsu, F. and Nasu, M.1996. PCR Detection of DNA specific for Aspergillus species in serum of patients with invasive aspergillosis. J. Clin. Microbiol., 34: 2464- 2468.

Zafra, R.1.; Pérez, J.; Pérez-Ecija, R .A.; Borge, C.; Bustamante, R.; Carbonero ,A. and Tarradas, C. 2008. Concurrent aspergillosis and ascites with high mortality in a farm of growing broiler chickens. Avian Dis., 52(4):711-713.

Zhao,Y. and Perlin, D. S. 2013. Quantitative detection of Aspergillus spp. by real time nucleic acid sequence- based amplification. Met. Mol. Biol., 968: 83-92.