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Mycological quality of some chicken meat cuts in Gharbiya governorate with special reference to *Aspergillus flavus* virulent factors

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ABSTRACT

Contamination with mold and yeast, and their toxins is considered one of the most dangerous hidden pollutants that threaten the health of the consumer. The presence of mycotoxins in various foods has been recorded, despite their apparent safety for human consumption. So, an investigation was conducted to evaluate the mycological quality, with special reference to the presence of toxigenic *Aspergillus* species, by culture method and aflatoxin producing genes molecularly in a total of 75 random samples of chicken cuts represented by wing, breast and thigh (25 of each) that were collected from various groceries and poultry shops located at Gharbiya governorate, Egypt. Results revealed that breast samples had the highest mycological counts (2.85 CFU/g). Consequently, isolated strains were subjected for mycological classification; where results revealed detection of *Aspergillus*, *Penicillium*, *Cladosporium*, *Rhizopus* and *Alternaria* species in various ratios in the examined samples. Moreover, *Aspergillus* species have been found in 36%, 48% and 40% of the examined wing, breast and thigh samples, respectively. Furthermore, mycological identification of the isolated *Aspergillus* strains showed the presence of *A. niger*, *A. flavus*, *A. fumigatus*, *A. terreus* and *A. parasiticus* in 16%, 13.3%, 10.6%, 1.3% and 1.3% of the total population of the examined samples. Molecular detection of some aflatoxin production regulating genes (*OmtA*, *Nor1* and *Ver1*) in ten *Aspergillus sp.* isolates revealed their detection in 8/10 (80%), 8/10 (80%) and 7/10 (70%) represented by positive bands at molecular weight of 1024 bp, 400 bp and 537 bp, respectively. Referring to the recorded results, chicken cuts may possess a great silent hazard to the human-being under improper good manufacturing practices and inadequate hygienic conditions during handling and storage.

1. INTRODUCTION

Chicken meat and meat products production in developing countries plays an essential role in supporting food security and poultry meat demands (Wong *et al.*, 2017).

Contamination of chicken meat products with molds can occur during different preparation stages during slaughtering under bad hygienic conditions using contaminated water or by adding contaminated spices with mold spores or during packing, handling, transportation and storage (Khalalfalla *et al.*, 2017).

Contamination of chicken meat with *Aspergillus* species, especially *Flavus* section, is one of the most hazardous microbial contaminations as the majority of *Aspergillus* species are able to produce aflatoxins that can cause diseases associated with aflatoxin poisoning and carcinogenic effects (Leggieri *et al.*, 2021).

Acute aflatoxin poisoning may lead to death as was recorded in Kenya in 2004 (Probst *et al.*, 2007), while chronic poisoning may lead to various recorded mutagens and cancers (Benkerroum, 2020).

Aspergillus sp. was classified into two groups depending on their toxigenic impacts on food and human health; 1st group

includes the aflatoxigenic species such as *A. flavus* and *A. parasiticus*, while the 2nd group contains the non-aflatoxin-producing species such as *A. tamarii* and *A. oryzae* (Frisvad *et al.*, 2019).

Molecular analyses have been used to confirm aflatoxin productivity of *Aspergillus* species isolates. *omtA*, *nor1* and *ver1* genes are from the commonly used genes encoded *aflP*, *aflD* and *aflM* toxins detection in food items (Sohrabi and Taghizadeh, 2018) yield an accurate, rapid and reliable records of toxigenic *Aspergillus* species especially in food chain (Sadhasivam *et al.*, 2017).

Therefore, the current study was conducted to evaluate the mycological quality with special reference to the presence of toxigenic *Aspergillus* species by culture method; and aflatoxin producing genes molecularly in total of 75 random samples of chicken cuts represented by wing, breast and thigh (25 of each) that were collected from various groceries and poultry shops located at Gharbiya governorate, Egypt.

2. MATERIAL AND METHODS

2.1. Collection of Samples:

A total of seventy-five random samples of raw chilled chicken wing, chicken thigh, chicken breast (25 of each) was

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collected from different local poultry shops and different supermarkets at Gharbia governorate, Egypt. Samples were taken aseptically in polyethylene bags and were transferred to the laboratory in an ice box for mycological examination.

2.2. Preparation of samples (ISO, 2017):

Twenty-five grams from each sample were carefully and aseptically homogenized in blender after mixing with 225 ml of sterile peptone water 0.1% to form a dilution of 1:10, from which tenth fold serial dilutions were prepared.

2.3. Determination of yeast and mold contamination:

2.3.1. Culture of the prepared samples:

was performed according to ISO (2008), where 0.1ml of the previously prepared serial dilutions was speeded by a sterile L-shape glass rod over two Petri-dishes contained solidified Dichloran Rose Bengal agar with chloramphenicol (DRBC) then were incubated at upright position at 25OC for 5 -7 days.

2.3.2. Identification of isolated strains:

was performed according to Pitt and Hocking (2009) macroscopically and microscopically as recorded in Table (1).

Table 1 Oligonucleotide primers sequences

Gene	Sequence (5'----3')	Amplified product (bp)	Reference
<i>omtA</i>	R GGCCCGGTTCTGGCTCCTAAGC	1024	Norlia <i>et al.</i> , 2019
	F CGCCCCAGTGAGACCCTTCCTCG		
	R ACCGCTACGCCGGCACTCTCGGCA		
<i>nor1</i>	F GTTGCCGCCAGCTTCGACTCC	400	Norlia <i>et al.</i> , 2019
	R GCCGCAGCCGGGAGAAAGTGG		
<i>ver1</i>	F TGGGATATACTCCCGCACACAG	537	
	R CC		

2.4. Molecular detection of some aflatoxin producing genes of some isolated *Aspergillus* strains by PCR:

2.4.1. Oligonucleotide primers used in PCR:

Three pairs of *omtA*, *nor1* and *ver1* primers were prepared and collected from Metabion (Germany). Their special sequence and amplify certain products as were displayed in Table (2).

2.4.2. Mycological DNA was extracted following QIAamp DNeasy Plant Mini kit Catalogue no. 691042.

2.4.3. Preparation of master mix and thermal profile was adapted according to the manufacturer instructions (Emerald Amp GT PCR mastermix (Takara) Code No. RR310A).

3. RESULTS

Referring to the recorded results in Table (2), breast samples had the lowest incidence of contamination; while thigh samples had the highest incidence of contamination with yeast and mold, followed by wing samples.

Table 2 Statistical analytical counts of yeast and mold (log10 CFU/gm) in the examined chicken meat cuts samples (N= 25 of each)

Samples	Prevalence of positive samples		Yeast and mold counts		
	No.	%	Min.	Max.	Mean ± S.E.
Chicken thigh	12	48	1.37	2.85	2.48±0.16
Chicken breast	5	20	1.88	2.97	2.85±0.01
Chicken wings	11	44	1.91	2.31	2.01±0.22

Regarding with the fitness of the examined samples for human consumption as recommended by EOS 3494/2005 standard; Table (3) showed that 37.3% of the examined

samples were considered to be unfit for human consumption because of contamination with yeast and mold.

Table 3 Fitness of the examined chicken meat cuts samples based on EOS standards (N= 25 of each)

Samples	MPL	Accepted		Rejected	
		No	%	No.	%
Chicken thigh	<10 CFU/g	13	52	12	48
Chicken breast	<10 CFU/g	20	80	5	20
Chicken wings	<10 CFU/g	14	56	11	44
Total	N= 75	47	62.7	28	37.3

Referring to the obtained results of microbiological typing of the isolated mold strains as tabulated in Table (4); five fungal species were detected. *Aspergillus* species was the most detected species (41.3%), followed by *Penicillium*, *Cladosporium*, *Rhizopus* and *Alternaria*, respectively.

Table 4 Typing of the isolated mold in examined chicken meat cuts samples (N= 25 of each)

Samples	Wings		breast		Thigh		Total	
	No	%*	No	%*	No	%*	No	%**
<i>Aspergillus</i>	9	36	12	48	10	40	31	41.3
<i>Penicillium</i>	5	20	3	12	7	28	15	20.0
<i>Cladosporium</i>	3	12	4	16	4	16	11	14.7
<i>Rhizopus</i>	2	8	3	12	-	-	5	6.7
<i>Alternaria</i>	-	-	2	8	3	12	5	6.7

Table (5) recorded the observed macroscopical and microscopical characters of the isolated *Aspergillus* species from the examined chicken meat cuts samples.

Table 5 Morphological character of *Aspergillus* species (Pitt and Hocking, 2009)

	Colony Diameter (mm)	Texture	Surface color	Reverse color	Stipes	Vesicles	Setation	Conidia	Colonial head/ chlosterobea
<i>flavus</i>	65-70	Floccose powdery or granular	Greenish yellow	Pale brown	Rough hyaline	Globose or subglobose	Biseriate	Globose to ellipsoid	Radiating head
<i>fumigatus</i>	40-70	Velvety to powder	Blue with white margin	Slight green	Smooth hyaline	Clavate	Uniseriate	Globose or subglobose	Columnar head
<i>nidulatus</i>	50-65	Velvety	Green	Brown	Smooth brown	Pyiform	Biseriate	Globose rough	Radiate Head/heads Cells axes
<i>niger</i>	50-70	Granular or powdery	Black	Pale yellow to brown	Smooth Yellow to brown	Round	Biseriate	Globose brown	Round head
<i>terreus</i>	40-60	Powdery	Sandy to brown	Pale brown	Smooth hyaline	Round to pyriform	Biseriate	Globose to ellipsoid	Columnar head

Regarding with the typing of the *Aspergillus* genera identification, *A. niger* had the highest detection levels (16%) in the examined samples (Table 6).

Table 6 Prevalence of identified aspergillus sp. in the examined chicken meat cuts (n= 25 of each)

Samples	wings		breast		thigh		Total	
	No.	%	No.	%	No.	%	No.	%
<i>Aspergillus</i> spp.								
<i>A. niger</i>	4	16	6	24	2	8	12	16
<i>A. flavus</i>	2	8	4	16	4	16	10	13.3
<i>A. fumigatus</i>	2	8	1	4	5	20	8	10.6
<i>A. terreus</i>	1	4	-	-	-	1	1.3	
<i>A. parasiticus</i>	-	-	1	4	-	1	1.3	

Referring to the obtained results of molecular detection of some aflatoxin producing genes as recorded in Table (7) and Figs (1 to 3); *omtA*, *nor1* and *ver1* genes were detected in

8/10 (80%), 8/10 (80%) and 7/10 (70%) of the examined *A. flavus* isolates, respectively. Presence of these genes indicated the producibility of the examined strain for aflatoxins P, D and M, respectively.

Table 7 Prevalence of aflatoxin producing genes in *A. flavus* isolates from the examined samples (n= 10)

Sample	<i>omtA</i>	<i>nor1</i>	<i>ver1</i>
1	+	+	+
2	-	-	-
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	-
8	-	-	-
9	+	+	+
10	+	+	+

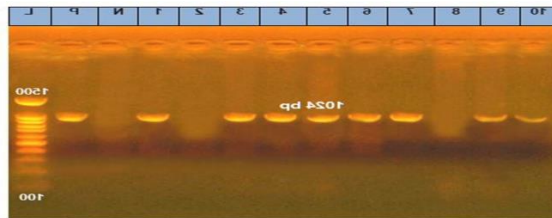


Figure 1 Agarose gel electrophoresis of cPCR of *omtA* (1024 bp) gene of *A. flavus*. Lane L: 100 bp ladder as molecular size DNA marker. Lane P.: Control positive *A. flavus* for *omtA* gene. Lane N.: Control negative. Lanes 1, 3, 4, 5, 6, 7, 9 and 10: Positive *A. flavus* for *omtA* gene. Lanes 2 and 8: Negative *A. flavus* for *omtA* gene.

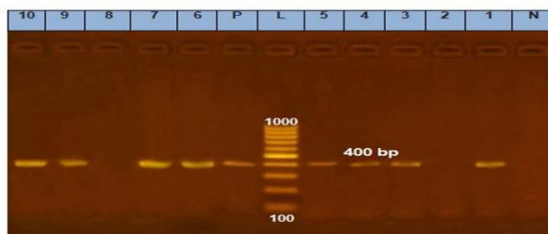


Figure 2 Agarose gel electrophoresis of cPCR of *nor1* (400 bp) gene of *A. flavus*. Lane L: 100 bp ladder as molecular size DNA marker. Lane P.: Control positive *A. flavus* for *nor1* gene. Lane N.: Control negative. Lanes 1, 3, 4, 5, 6, 7, 9 and 10: Positive *A. flavus* for *nor1* gene. Lanes 2 and 8: Negative *A. flavus* for *nor1* gene.

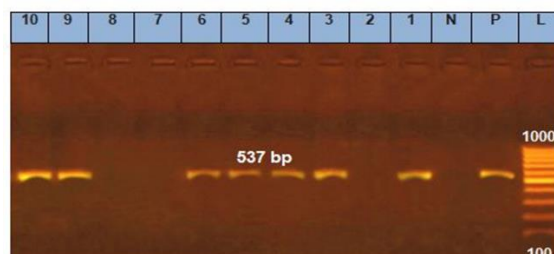


Figure 3 Agarose gel electrophoresis of cPCR of *ver1* (400 bp) gene of *A. flavus*. Lane L: 100 bp ladder as molecular size DNA marker. Lane P.: Control positive *A. flavus* for *ver1* gene. Lane N.: Control negative. Lanes 1, 3, 4, 5, 6, 9 and 10: Positive *A. flavus* for *ver1* gene. Lanes 2, 7 and 8: Negative *A. flavus* for *ver1* gene.

4. DISCUSSION

Chicken meat and meat products comply an important source of human protein supplement all over the world because they provide good source of digestible protein, low cholesterol fat, essential amino acids, minerals, and different types of vitamins and minerals.

In Egypt, as well as human population increasing, demand of animal proteins also is increasing represents a serious challenge in which poultry industry plays an essential role in filling nutrition gap as a rapid and more economic source of proteins (Shaltout *et al.*, 2015).

Mold contamination of meat and meat products have been considered a serious source of food spoilage resulting in different organoleptic changes in flavor, color, texture, odor referred mainly to the fungal deterioration especially in poor developing countries due to lack of hygienic measures during processing and handling (Lorenzo *et al.*, 2018).

Presence of mold in foods may be referred to the rapid, easy disperse and wide spread of the fungal spores which are abundant in the environment introducing food chain through dust, water, workers and equipment. Their presence in food samples is a serious public health concern as these fungi may be associated with the production of mycotoxins (Benedict *et al.*, 2016).

Referring to the obtained results of yeast and mold counts, higher results were recorded by Eldaly *et al.* (2002) (3.36 log₁₀ CFU/g), while nearly similar results were reported by Shaltout *et al.* (2016) (2.8 and 2.4 log₁₀ CFU/g for mold and yeast, respectively), and Hassan (2019) (2.2 log₁₀ CFU/g).

In addition, mycological classification of the detected fungi was compatible with those reported by Shaltout *et al.* (2016), Hassan *et al.* (2019) and Abuzaid *et al.* (2020) who detected *Aspergillus* spp., *Acremonium* spp., *Cladosporium* spp., *Penicillium* spp.; among which *Aspergillus* species was the most prominently detected in their examined samples. Moreover, they recorded isolation and identification of *A. niger*, *A. flavus*, *A. fumigatus*, *A. parasiticus* and many other genera with variations which came in line with the current obtained results. Furthermore, *Aspergillus* sp. was prominently detected in breast samples other than wings and thighs samples, which came in agree with the previously recorded results of Darwish *et al.* (2016) and Shaltout *et al.* (2019) who found that the examined breast samples were more contaminated with fungal infection than wing and thigh samples. While the current prevalence of *Aspergillus* species in the examined samples came lower than those recorded by Hassan (2019) who found *Aspergillus* sp. in all the examined thigh samples (100%) collected from Gharbiya governorate, Egypt. Moreover, Abuzaid *et al.* (2020) also detected *A. flavus* and *A. niger* in 40% and 80% of the examined sausage samples of chicken origin, respectively. Variations between different authors may be attributed to difference in sources of samples collections and hygienic quality of production plants.

Aspergillus species represents an important mycotic infection in public health concern as a human pathogen and as toxin-producing food contaminant. It releases a lot of spores which found in air, water, soil, plant debris, manure and animal feed. As fungal spore's growing, it secretes digestive enzyme and mycotoxins leading to food spoilage and human mycotoxicosis (Richardson and Rautemaa-Richardson, 2019).

Referring to the obtained results of the mycological identification of *Aspergillus* sp. isolates as recorded in Table

(6), they came in agree with the previously reported results by Darwish *et al.* (2016) who found that *A. niger* was the predominant detected strain, followed by *A. flavus* and *A. parasiticus* in the examined samples of chicken cuts collected from Zagazig city, Egypt.

Some mold species can cause respiratory infections representing a significant risk for individual with severely weakened immune system (OSHA, 2010). Presence of mold in high incidence indicate bad hygienic measures adopted during handling, preparation and processing (El-Abbasy, 2007).

Mycotoxins have been defined as naturally occurring secondary fungal metabolites produced in meat and meat products by direct growth of toxigenic molds such as *Aspergillus* species which produce aflatoxins and ochratoxins which threat public health due to their carcinogenic, hepatotoxic, nephrotoxic, teratogenic and mutagenic effects in human and animals (Agriopoulou *et al.*, 2020).

Aflatoxins are produced by a polyketide pathway that pass through about twenty-seven enzymatic reactions which have been regulated by sets of genes including *nor-1*, *ver-1* and *omtA* have been shown to be involved in this process. *aflD* (*nor-1*) encodes a norsolorinic acid ketoreductase needed for the conversion of the 10-keto group of Norsolorinic Acid (NOR) to the 10-hydroxyl group of Versicolorin A (VERA) (Zhou and Linz, 1999). *aflM* (*ver-1*), predicted to encode a ketoreductase, is involved in the conversion of VERA into Sterigmatocystin (ST) (Henry and Townsend, 2005); *aflP* (*omtA*) codes for O-methyltransferase, which is one of the main genes responsible for transforming ST into O-methylsterigmatocystin (OMST) that is the precursor for aflatoxin production (Yabe *et al.*, 1989).

Many other previous studies recorded detection of these genes in their *Aspergillus* isolates of food origin by various PCR techniques; Manonmani *et al.* (2005), Rodrigues *et al.* (2009), and Hassan *et al.* (2015), who conducted several studies investigating the aflatoxigenicity of *Aspergillus* sp., could detect different genes in their *Aspergillus* isolates.

5. CONCLUSION

It could be concluded that, breast samples revealed the highest contamination levels with *Aspergillus* sp.; in addition, *A. niger* was the prominently detected strain. PCR technique is a unique diagnostic tool for detection and identification of aflatoxigenic *Aspergillus* strains especially if the field of food safety. So, application of strict hygienic measures, proper use of water supply and food additives from good sources is recommended.

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