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Research Article

Molecular Detection of Aflatoxin Producing Aspergillus Species Isolates in Some Chicken Meat Cuts In Gharbiya Governorate, Egypt

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Abstract

Contamination with fungi 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. Therefore, the current study was conducted to evaluate the prevalence of 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 poultries shops located at Gharbiya governorate, Egypt. Results of culture and isolation techniques revealed detection of Aspergillus sp. in 36, 48 and 44% of the examined wing, breast and thigh samples, respectively. Moreover, microbiological identification of the isolated strains showed 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, 400 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.

Keywords: aspergillus species; chicken meat cuts; cpcr; egypt

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 meat products with molds can be 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 meat with Aspergillus species, especially Flavi section, is one of the most hazardous microbial contamination as the majority of Aspergillus species are able to aflatoxins production 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 main target of the current study was to investigate the presence of toxigenic aspergillus species in some chicken meat cuts collected from Gharbiya Governorate markets, Egypt.

Material and Methods

Collection of samples

A total of seventy-five random samples of raw chilled chicken wing, chicken thigh and chicken breast (25 of each) were collected from

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

Preparation of samples (ISO, 2017)

Twenty-five grams from each sample were carefully and aseptically homogenized in blinder 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.

Determination of Aspergillus species

culture of the prepared samples was performed according to **ISO (2008),** where 0.1ml of the previously prepared serial dilutions was spreaded by mean of 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 25^{O}c for 5 -7 days.
Identification of isolated strains was performed according to **pitt and hocking (2009)** macroscopically and microscopically as recorded in **Table (1).**

Molecular Detection Of Some Aflatoxin Producing Genes Of Some Isolated Aspergillus Strains By Cpcr

Oligonucleotide primers used in cpcr

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

Mycological DNA was extracted following QIAamp DNeasy Plant Mini kit Catalogue no. 69104
Preparation of master mix and thermal profile was adapted according to the manufacturer instructions (Emerald Amp GT PCR mastermix (Takara) Code No. RR310A).

	Colony Diameter (mm)	**Texure**	**Surface color**	**Reverse color**	**Stipes**	**Vesicles**	**Serriation**	**Conidia**	**Colonial head/ cleistotheca**
flavus	65-70	Floccose powdery or granular	Greenish yellow	Pale brown	Rough hyaline	Globose or subglobose	Biseriate	Globose to ellipsoid	Radiating head
fumaiguts	40-70	Vevety to powder	Blue with white margin	Slight green	Smooth hyaline	Clavate	Uniseriate	Globose or subglobose	Columner head
nidulans	50-65	Velvety	Green	Brown	Smooth brown	Pyriform	Biseriate	Globose rough	Radiate Head/hulla Cells ascs
niger	50-70	Granular of powdery	Black	Pale yellow	Smooth Yellow to brown	Round	Biseriate	Globose brown	Round head
terus	40-60	Powdery	Sandy to brown	Pale brown	Smooth hyaline	Round to pyriform	Biseriate	Globose to ellipsiod	Columner head
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Table 1: *Morphological character of Aspergillus species (Pitt and Hocking, 2009)*

Gene	Sequence (5' ---3')	Amplified product (bp)	Reference
omtA	GGCCCGGTTCCTTGGCTCCTAAGC	1024	Norlia *et al.*, 2019
	CGCCCCAGTGAGACCCTTCCTCG		
nor1	ACCGCTACGCCGGCACTCTCGGCAC	400	
	GTTGGCCGCCAGCTTCGACACTCCG		
ver1	GCCGCAGGCCGCGGAGAAAGTGGT	537	
	GGGGATATACTCCCGCGACACAGCC		

Table 2: *Oligonucleotide primers sequences*

Results

As recorded in **Table (3);** Aspergillus sp. was detected in 32(42.6%) of the total examined samples. In detail, breast samples recorded the highest contamination level (48%); followed by thigh and wing samples, respectively.

Samples	Wing		Breast		Thigh		Total	
	No.	%*	No.	%*	No.	%*	No.	%**
Aspergillus sp.	9	36	12	48	11	44	32	42.6

Table 3: *Prevalence of Aspergillus species in the examined chicken meat cut samples (n= 25 of each).*

%* prevalence in relation to the number of each sample (25)

%** prevalence in relation to the total number of samples population (75).

Regarding with the genus identification, *A. niger* had the highest detection levels (16%) in the examined samples **(Table, 4).**

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

Table 4: *Prevalence of identified aspergillus sp. in the examined chicken meat cuts (n= 25 of each)*

Referring to the obtained results of molecular detection of some aflatoxin producing genes as recorded in **Table (5) 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.

Sample	*omtA*	*nor1*	*ver1*
1	+	+	+
2	-	-	-
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	-
8	-	-	-
9	+	+	+
10	+	+	+

Table 5: *Prevalence of aflatoxin producing genes in A. flavus isolates from the examined samples (n= 10)*

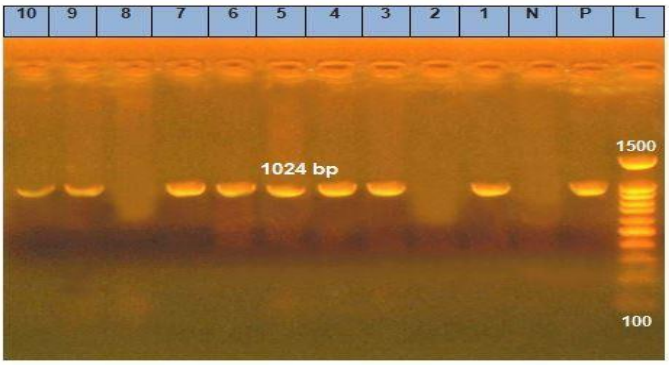

Figure 1: Agarose gel electrophoresis of cPCR of *omtA* (1024 bp) gene of *A. flavus.*

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.

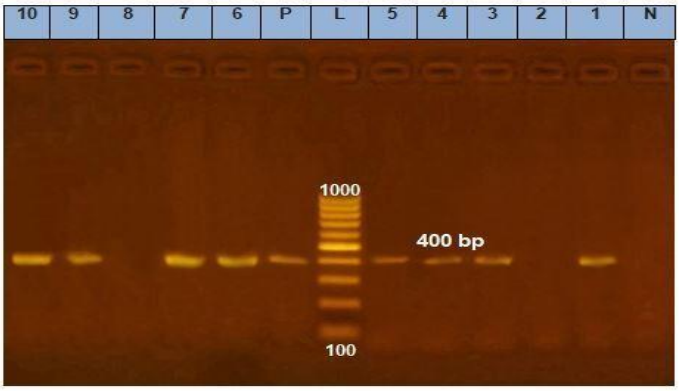

Figure 2: Agarose gel electrophoresis of cPCR of nor1 (400 bp) gene of *A. flavus.*

Lane L: 100 bp ladder as molecular size DNA marker.
Lane P.: Control positive *A. flavus* for *nor*1 gene.
Lane N.: Control negative.
Lanes 1, 3, 4, 5, 6, 7, 9 and 10: Positive *A. flavus* for *nor*1 gene.
Lanes 2 and 8: Negative *A. flavus* for ***nor*1** gene.

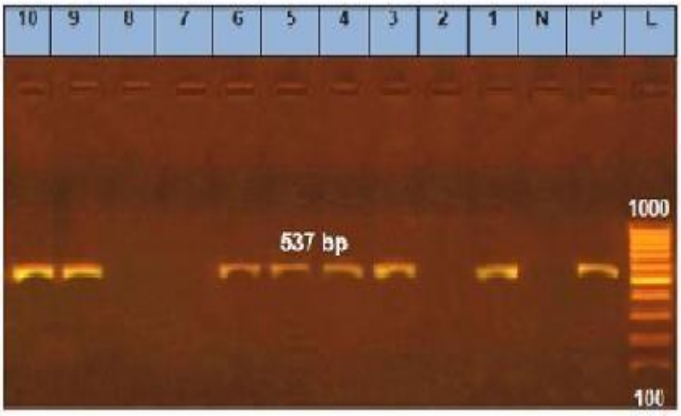

Figure 3: *Agarose gel electrophoresis of cPCR of ver1 (400 bp) gene of A. flavus.*

Lane L: 100 bp ladder as molecular size DNA marker.

Lane P.: Control positive *A. flavus* for ***ver*1** gene.

Lane N.: Control negative.

Lanes 1, 3, 4, 5, 6, 9 and 10: Positive *A. flavus* for ***ver*1** gene.

Lanes 2, 7 and 8: Negative *A. flavus* for ***ver*1** gene.

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).**

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 recorded results in **Table (3),** 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 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.

Referring to the obtained results of the microbiological identification of Aspergillus sp. isolates as recorded in **Table (4),** 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 are 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.

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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