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Prevalence of toxigenic *Aspergillus flavus* in meat and meat products.

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Contamination of meat and meat products with toxigenic *Aspergillus flavus* is of major public health concern as this fungus is capable of production of extremely dangerous toxins known as aflatoxins when suitable conditions of humidity and temperature are available. Aflatoxin B_1, B_2, G_1 and G_2 are considered the four major types of aflatoxins however aflatoxin B_1 is the most dangerous one and it is known as the most powerful naturally occurring toxin. A total of one hundred and twenty samples of meat (40 samples) and meat products which include luncheon (40 samples) and hamburger (40 samples) were mycologically examined and the total yeast and mold count were performed using the pour platting technique whereas the toxigenic *Aspergillus flavus* strains were detected via thin layer chromatography (TLC) technique. The total yeast count / gm for meat, luncheon and beef burger was calculated with mean ± standard $6.8\times10^2 \pm 1.3\times10^2$, $2.9\times10^3 \pm 7.2\times10^2$ and $4.3\times10^3 \pm 1.1\times10^3$, respectively while the total mold count was $5.58\times10^4 \pm 2.96\times10^4$, $4.3\times10^2 \pm 6.7\times10$, $3.4\times10^3 \pm 7.0\times10^2$ and $1.5\times10^3 \pm 3.0\times10^2$, respectively. The present study assured that the most commonly isolated fungal genera were *Aspergillus* (75.8%). Among *Aspergillus* genus, *Aspergillus flavus* was the most frequently isolated species as it was isolated from (31.7%) from total samples. The incidence of toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* was also detected and the results revealed that 18 (47.4%) out of 38 *Aspergillus flavus* isolates were toxigenic while they were 2 (66.7%) out of three for *Aspergillus parasiticus* isolates. The prevention of mould contamination in the meat of slaughtered animals, in all the ingredients used especially spices, as well as in the manufacturing rooms, stores, and shops is crucial in order to prevent mycotoxins production.

Keywords: Meat, Meat products, Total yeast count, Total mould count, toxigenic *Aspergillus flavus*.

INTRODUCTION

Meat and meat products are important sources of food for the human being as they are considered the most concentrated and easily assimilated nitrogenous foods Ahmed (1995).

Contamination of meat with fungi originally came from the intestinal content of the slaughtered cattle together with the increase of bad hygienic conditions during handling of carcass Farghaly et al., (2001). In addition to that, Atmosphere of the abattoirs in which the animal were slaughtered and the ripening chambers can be considered an important source of fungal infection. Annadurai et al., (2003) and Comi et al., (2004).

In meat products, Mould contamination may be originated from meat or from the addition of low quality flavoring agents, spices, in particular and inappropriate hygienic conditions during handling, processing and storage of the products

can increase the fungal load Gourama and Bullerman (1995). Moreover, Contamination with Moulds can lead to a great economic losses and also moulds considered a major producer of mycotoxins Abdel-Rahman and El-Khatib (1989*)*. luncheon contamination with aflatoxins may be originated from either animal tissues previously fed on aflatoxin contaminated feed or due to using contaminated components like cereals and spices Zaky et al., (1995) .

Frequent presence of moulds in meat indicate the bad hygienic measures adopted in the slaughter houses, in processing and handling of fresh meat El-Daly et al., (1988).

Contamination with yeasts and moulds in foods and feed samples can lead to deterioration and toxins production Dabirian (2005).

Aspergillus flavus is well known to be the main producer of the carcinogenic aflatoxins. The existence of this fungus and their toxins is of great importance in terms of food safety Rodrigues et al., (2007).

Humans are exposed to aflatoxin directly by consuming contaminated food or indirectly by consuming animal products previously fed on aflatoxins contaminated feeds Brr et al., (2004).

Aflatoxins are well known for their toxic and carcinogenic impacts on both humans and animals Cervino et al., (2007).

MATERIALS AND METHODS

Samples

A total of one hundred and twenty samples of meat (40 samples) and meat products which include luncheon (40 samples) and hamburger (40 samples). Those samples were obtained from different shops in Cairo and Giza governorate.

Isolation, identification and count of fungi from the tested samples

Preparation of sample homogenate

twenty five grams of each sample were aseptically homogenized in a stomacher bag containing 225 ml of 1% sterile buffered peptone water and mix it in stomacher for 30-60 seconds to give 0.1 dilution.

b-Serial dilution

Pipette 1ml of food homogenate into a tube containing 9 ml of the diluent. From the first dilution transfer 1 ml to the second dilution tube containing 9 ml of the diluent and so on until the desired dilution was obtained**.**

c-Pour plating

Pipette 1 ml of the sample homogenate and of such dilutions which have been selected for plating into a petri dish in duplicate. Pour into each Petri dish 10 to 20 ml molten SDA (cooled to 42-45 ℃). Mix the media and dilutions by swirling gently clockwise and anti-clockwise and allow setting**.**

Incubation

Inoculated plates were left to solidify at room temperature. The plates were inverted to prevent spreaders and incubated at 25 ℃ for 3-5 days. The yeast colonies which were dull white, creamy, yellow, pink, regular and irregular shape were counted separately using a colony counter and yeast count/gram was calculated and recorded and for mould at 25 ℃ for 5-7 days at an inverted position. During the incubation period, the plates were examined daily for the star shaped mould growth **(**American Public Health Association, 1992)**.**

Identification of isolated moulds: Samson et al., (2010)

Screening of A.flavus and A. parasiticus isolated from tested samples for aflatoxins production in YES medium

Growth of *A.flavus* and *A. parasiticus* on YES medium

Isolated strains of *A. flavus* and *A. parasiticus* were inoculated into YES medium and incubated at 28ºC for 15 days. After the end of the incubation period, Filtrate the medium with what man filter paper no. 1 to remove mycelial mat then the toxin was extracted from the filtrate by refluxing it against equal volume of chloroform then the chloroform layer was drained and passed over a thin layer of 10 gram of anhydrous sodium sulphate. The extract was evaporated till complete dryness.

Determination of aflatoxin residues using TLC technique AOAC (1995)

Preparation of aflatoxin standard solution

Benzene - acetonitrile (9-1) was added to the container of dry aflatoxin and the concentration calculated to give 8-10µg/ml. the solution was agitated for one minute and transferred into a glass stopered flask. By using the automatic pipette a portion of the stock standard aflatoxin solution was diluted with benzene- acetonitrile (9-

1) to obtain a concentration of 0.5 µg aflatoxin B_1 and the flask containing the stock solution was weighed, wrapped tightly in aluminum foil and stored at 0 ºC till used.

Detection of aflatoxins by TLC:

Resolutions of reference aflatoxin B_1 solution was prepared to give a final dilution with Benzene - acetonitrile (9:1) 0.5 µg aflatoxin B_1.

A vial of sample extract residue was uncapped and 0.1 µl Benzene - acetonitrile (9:1) was added and mixed. Activation of thin layer plates for one hour in hot air oven at 110 ºC and it was removed to cool.

A known volume of the sample solution spots of (5, 10, 20 and 40 µl) was spotted on an imaginary line from the bottom edge of the plate. Standard solution was spotted on the plate with known concentration using 10-20 µl capillary pipettes.

The plates was developed with chloroform: acetone 9:1 (V/V) in an equilibrate jar or developing tank for 30 minutes. When the solvent travels about 12 cm front, the plates were removed from the jar, air dried and inspected under long wave ultraviolet light lamp (360 nm) for examining the tested and standard spots matches and aflatoxins spots which emit blue fluorescence under ultraviolet light on the TLC glass-silica gel plates.

Statistical analysis:

Data obtained were analyzed statistically for descriptive statistics (mean, maximum minimum and standard error) using SPSS 14.

RESULTS AND DISCUSSION:

The contamination with *Aspergillus* is not only of economic importance but also represents a real health hazard. It has allergic, toxigenic and pathogenic impact through the production of mycotoxins Brr et al., (2004). Some species of *penicillium* were found to be associated with pulmonary and urinary tract infections in addition to yellow "rice disease" causing many deaths in man Washington 1981. while *Mucor* and *Rhizopus* species may cause lesions in lungs, skin, eye, ear, gastrointestinal tract also cellulitis and deep wound infections can occur Jawetz et al., (1974) and Banwart (1980). *Cladosporium* species also can cause chromatomycosis and brain abscesses Edris (1986) and Jawetz et al., (1974). *Fusarium* species may induce mycotic keratitis and skin infection Banwart (1980) and Jawetz et al., (1974).

In the current study, A total of one hundred and twenty samples of meat (40 samples) and meat products which include luncheon (40 samples) and hamburger (40 samples), were subjected to a mycological examination that revealed the recovery of yeasts from 47.5%, 65% and 77.5%, respectively and from 50%, 60% and 67.5% in case of moulds and our results were lower than those obtained by Ismail et al., (2013) as they isolated mould and yeast in the examined Luncheon samples and it were 23 (92%) and 7 (28%), respectively and also Samaha (2013) as he detected molds in 92% of meat samples.

To ensure a good hygienic condition of feed samples, the total fungal counts of samples must not exceed the value suggested as a limit which is (1×10^4 cfu / gm). Those high levels could decrease the nutrient adsorption Ogundero (1987) and palatability Martins and Martins (2001).

Table (1) demonstrated the total yeast count /gm for meat, luncheon and beef burger and it was calculated with min. 3X10, $1.2X10^2$ and $1.7X10^2$, whereas the max. $1.8X10^3$, $1.1X10^4$ and $1.6X10^4$ and finally the mean ± standard error was $6.8X10^2 \pm 1.3X10^2$, $2.9X10^3 \pm 7.2X10^2$ and $4.3X10^3 \pm 1.1X10^3$, respectively.

It is clear that beef burger samples contained the highest count followed by luncheon samples and finally meat samples. Beef burger samples range was from $1.7X10^2$ to $1.6X10^4$ and those results were nearly similar to the results obtained by Mousa et al., (2014). In luncheon samples, they ranged between $1.2X10^2$ and $1.1X10^4$ and the obtained results came in concordance with others reported by Ismail (1995) and were higher than those of Abou Arab (1995) and were lower than the results of El-Tabiy (2006) but meat sample ranged from 3X10 and $1.8X10^3$ which was to some extend similar with Oyero and Oyefolu (2010) and lower to those of Samaha (2013).

As shown in Table (2), The total mould count / g in meat, luncheon and beef burger was determined with min..

Table (1): Statistical analysis of total yeast count / g in meat and meat products.

Types of examined samples	Total yeast count /gm			
	Min.	Max.	Mean	± SE

Meat	3×10	1.8×10^3	6.8×10^2	1.3×10^2
luncheon	1.2×10^2	1.1×10^4	2.9×10^3	7.2×10^2
Beef burger	1.7×10^2	1.6×10^4	4.3×10^3	1.1×10^3

Table (2): Statistical analysis of total mould count in meat and meat products.

Types of examined samples	Total moulds count (TMC) /gm			
	Min	Max	Mean	± SE
Meat	2.00×10	1.3×10^3	4.3×10^2	6.7×10
luncheon	4.00×10	1.1×10^4	3.4×10^3	7.0×10^2
Beef burger	4.5×10	7.3×10^3	1.5×10^3	3.0×10^2

2.00X10 , 4.00X10 and 4.5X10, whereas the max. $1.3X10^3$, $1.1X10^4$ and $7.3X10^3$ and finally the mean ± standard error was $4.3X10^2$ ± 6.7X10, $3.4X10^3$ ± $7.0X10^2$ and 1. $5X10^3$±3. $0X10^2$, respectively. The meat samples results were in agreement with the results of Oyero and Oyefolu (2010) but in case of luncheon samples the results found to be higher than those of Mousa et al., (2014) whereas in beef burger samples, our results were higher than those of Brr et al., (2004) as the mean values of mould count were $9.12x10^2$ ± 2.01x $l0^2$ ·The variation of mould count in samples may be due to different levels of hygiene during manufacturing and storage.

A total of 265 mould strains belonging to 8 genera were isolated and identified from meat and meat products samples. The results given in Table (3) showed that the most commonly isolated mould genera in meat samples were *Aspergillus* (65%), *Penicillium* (22.5%), *Rhizopus* (5%), *Alternania* (5 %), *Cladosporium* (2.5%), *Mucor* (2.5%) and *Fusarium* (2.5%) and those results were in accordance with other results obtained by Oyero and Oyefolu (2010) as they revealed the isolation of 8 genera, namely, *Aspergillus*, *Alternaria*, *Fusarium*, *Cladosporium*, *Penicillium*, *Neurospora*, *Rhizopus* also Samaha (2013) assured the isolation of the following mould genera *Aspergillus* species (73.9%), *Penicillium* species (56.5%), *Cladosporium* species (51.1%), *Rhizopus* species (44.6%), *Mucor* species (39.1%), *Alternaria* species (34.8%).

In case of luncheon samples, the most commonly isolated mould genera were *Aspergillus* (75%), *Penicillium* (27.5%), *Cladosporium* (25%), *Rhizopus* (7.5%), *Alternania* (7.5 %), *Mucor* (5%), *Fusarium* (5%) and *Scopulariopsis* (2.5%) also El-Tabiy (2006) indicated that the commonly isolated mould genera belonged to *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Cladosporium* and *Mucor*. Ismail et al., (2013) agreed with our results as they identified 7 mould genera. The identified mould genera were *Aspergillus*, *Penicillium*, *Eupencillium*, *Eurotium*, *Mucor*, *Cladosporium* and *Byssochlamys nivea*.

Beef burger samples revealed the presence of *Aspergillus* spp. in a high percentage as *Aspergillus* spp.(87.5%), *Penicillium* (25%), *Alternania* (12.5 %), *Scopulariopsis* (10%), *Cladosporium* (7.5%), *Mucor* (7.5%), *Rhizopus* (2.5%) and *Fusarium* (2.5%) and those results were in agreement with Brr et al., (2004) as they found that *Aspergillus* and *Penicillium* species were the most prevalent isolated species followed by *Alternaria*, *Cladosporium*, *Fusarium*, *Mucor*, *Rhizopus*, *Sporotricum* and *Thamnidium* species were recovered at lower percentages. Among *Aspergillus* genus, *A. flavus* was the most frequently isolated contaminant as it was isolated from 71.7% from total cattle feed samples as shown in Table (4). Other fungal strains were *A. niger, A. fumigatus, A. terreus, A. candidus, A. ochraceus* and *A. parasiticus* and were isolated up to 58.3, 41.7, 16.7, 13.3, 5, 3.3 from the samples, respectively. Those results were in concordance with other results obtained by Mngadi et al., (2008) and Rosa et al., (2008) along with Diaz et al., (2008) as they isolated *A. flavus* in the rate of 34% from total isolated *Aspergilli* also Krnjaja et al., (2013) found that *Aspergillus flavus* was the most common species of *Aspergillus* genus by 36.69% however Khosravi et al.,(2008) isolated *Aspergillus flavus* from 48% of the tested samples furthermore Trung et al., (2008) observed that *A. flavus* isolated from more than 90% of the their samples.

For meat samples, *A. niger* was the most commonly isolated *Aspergillus* spp. followed by *A. fumigatus, A. flavus, A. ochraceus A. candidus* and *A. parasiticus* were isolated up 30, 27.5, 20, 5, 2.5, 2.5 % from the total samples, respectively.

Table (3): Incidence of the most commonly isolated mould genera from meat and meat products samples (n=120)

Mould Genera	Meat (No. 40)		luncheon (No. 40)		Beef burger (No. 40)		Total (No. 120)	
	No.	%	No.	%	No.	%	No.	%
Aspergillus	26	65	30	75	35	87.5	91	75.8
Penicillium	9	22.5	11	27.5	10	25	30	25
Rhizopus	2	5	3	7.5	1	2.5	6	5
Cladosporium	1	2.5	10	25	3	7.5	14	11.7
Alternania	2	5	3	7.5	5	12.5	10	8.3
Mucor	1	2.5	2	5	3	7.5	6	5
Fusarium	1	2.5	2	5	1	2.5	4	3.3
Scopulariopsis	0	0	1	2.5	4	10	5	4.2

Table (4)

Incidence of *Aspergillus species* in meat and meat products.

Aspergillus species	Meat (No. 40)		luncheon (No. 40)		Beef burger (No. 40)		Total (No. 120)	
	No.	%	No.	%	No.	%	No.	%
A. flavus	8	20	14	35	16	40	38	31.7
A. niger	12	30	7	17.5	13	32.5	32	26.7
A. fumigatus	11	27.5	1	2.5	6	15	18	15
A. terreus	0	0	1	2.5	5	12.5	6	5
A. candidus	1	2.5	1	2.5	3	7.5	5	4.2
A. ochraceus	2	5	3	7.5	0	0	5	4.2
A. parasiticus	1	2.5	0	0	2	5	3	2.5

Table (5): Incidence of toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* in meat and meat products.

Type of examined samples	No. of examined samples	Tested *Aspergillus* Species	Total no. of examined *Aspergillus* isolates	+ve toxigenic isolates	
				No.	%
Meat	40	*A. flavus*	8	4	50
		A. parasiticus	1	1	100
luncheon	40	*A. flavus*	14	8	57.1
		A. parasiticus	0	0	0
Beef burger	40	*A. flavus*	16	6	37.5
		A. parasiticus	2	1	50

Our results were to some extend in agreement with those reported by Oyero and Oyefolu (2010) who assured that the predominant genus identified were 7 species, these included *A. niger, A. tamari, A. fumigatus, A. terreus, A. flavus, A. citrinum,* and *A. sydowii.*

For luncheon samples, *A. flavus* was the most commonly isolated *Aspergillus* spp. followed *by A. niger, A. ochraceus A. fumigatus, A. terreus* and *A. candidus* were isolated up 35, 17.5, 7.5, 2.5, 2.5, 2.5 % from the total samples, respectively. These results were nearly similar to results obtained by El-Tabiy (2006) as he stated that *A. flavus* and *A. parasiticus* were the most predominant isolated spp.

For Beef burger samples, *A. flavus* was the most frequently isolated *Aspergillus* spp. followed by *A. niger A. ochraceus A. fumigatus, A. terreus* and *A. candidus* and were isolated up 40, 32.5, 15, 12.5, 7.5, 5 % from the total samples, respectively. Brr et al., (2004) also noted that *A. flavus*, *A. fumigatus* and *A. niger* were the most prevalent *Aspergillus* isolated.

The screening of samples for viable fungi is a useful practice in itself as it does not only act as an indicator for contamination but also supports the analysis of mycotoxins that could be present Rosa et al., (2008).

In the present work, The incidence of toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* was detected in meat and meat products and the results as shown in Table (5) revealed that 18 (47.4%) out of 38 *Aspergillus flavus* isolates were toxigenic while they were 2 (66.7%) out of three for *Aspergillus parasiticus* isolates. Those results were to some extend in agreement with other results obtained by Farghaly (1998) as he confirmed that 47.7% of Aspergillus flavus isolates obtained from 100 random swabs samples obtained from different meat cold stores were aflatoxin producer while Aziz and Youssef (1991) assured that 24 isolates of *Aspergillus flavus* and 16 isolates of *Aspergillus parasiticus* out of 150 samples of meat products and 100 samples of spices were the predominant aflatoxin producing moulds isolated from both processed meat products and spices also Cventnic and Pepeljnjak (1995) tested the aflatoxin producing ability of 75 strains of *A. flavus* and *A. parasiticus* isolated from 420 samples of smoked-dried meat products and they found that in regard to sequential method of aflatoxin determination, 5 of 8 isolates were detected in the first step using aflatoxin-producing ability medium - APA and all of them in the second step using extraction method from syntheses on moist shredded wheat - SW.

CONCLUSION

Prevention of mould development in the meat of slaughtered animals, as well as in the manufacturing rooms, stores, and shops is of great importance in order to avoid the risk of mycotoxin production. Mižáková et al., (2002) and the most effective mean to prevent aflatoxigenic moulds contamination of meat products is through application of strict hygienic measures during the processing of meat products and using a good quality flavoring agents as spices, as well as application of HACCP system during dealing with meat and during different production phases of the products. Educational programs and training courses must be adopted for meat handlers and workers.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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