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Molecular characterization of microorganisms isolated from some meat products and estimation of total aflatoxin

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Abstract

The occurrence of food poisoning represents the most important popular problem in developing countries. Fungi and bacteria are responsible for most frequently food poisoning as mycotoxigenic *Asperagillus flavus*, and *staphylococcus aureus.* As well as food-borne disease caused by *Escherichia coli* and *Salmonella***.** As the Conventional methods for detection of *food poisoning bacteria* and *fungi* in foods are generally time-consuming and labor intensive. The PCR method has been developed with custom designed primers for rapid, sensitive, less costely and specific detection method for these pathogens and its toxin genes in food. In addition, the method can reduce the time required for confirmation of isolates by up to 3 to 4 days compared with traditional methods.

Keywords: *Meat products, food poisoning, Asperagillus flavus, staph. aureus, Salmonella, E. coli, PCR.*

Introduction

Foodborne pathogens causing toxins-associated diseases are important health problems throughout the world in both developed and developing countries (**Gustafson *et al.,* 2014**). Several mycotoxins reported to date are cosmopolitan in distribution and incur severe health-associated risks (including cancer and neurological disorders) **(Bezerra *et al.,* 2014).** However processed meat products may carry contaminants that constitute a public health hazard as food borne pathogenic bacteria which cause illness, intoxication and sometimes outbreak of death. (**Rao *et al.,* 2009**). There has been increase in the production and consumption of processed meat products over all the world due to their low price, as well as, they represent quick ,easy prepared meat meals and solve the problem of storage in fresh meat of high price (**Mohamed, 2006**). On the other side, meat products are liable to harbor different types of M.O. through a long chain of handling, processing, distribution and storage as well as preparation (**Hassanin, 2004**). Family *Enterobacteriaceae* have playing a major role in the food poisoning and many of this illness are due to growth of pathogens and /or toxin formation as *Salmonella*, *Escherichia coli* and *Staphylococcus aureus*. (**Koka and Weimer., 2001** and **Ajoke *et al.,* 2014)**. Food borne diseases caused mainly by *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus* are the major causes of mortality and infections espicaily in the developing countries. This pathogens are transmitted mainly through consumption of contaminated food and the presence of those organisms in meat and meat products have relevant public health implications (**Zafar *et al.,* 2016**). The ability of *Salmonella* strains to cause disease is attributed to arrays of virulence genes defined in the *Salmonella* Pathogenicty Islants (SPIs), There are at least 60 genes associated with SPI, Different virulence genes such as (invA, stn, fumH, and hilA) are the major genes responsible for virulence factor in *Salmonella* (**Blum *et al*., 1994**). *Escherichia coli* considered as a normal common M.O. in the alimentary tract of most animals and human, which is a good indicator of possible fecal contamination (**Synge, 2000**), it com-

monly non virulent but some strains have a pathogenic or toxinogenic virulence factor and become pathogenic to human and animals (**Datta *et al.,* 2012).** *Staphylococus aureus* is considered t an important cause of human illness subsequent to ingestion of bacterial contaminated food In U.S. *Staph aureus* is represented 25% of all food illness. (**Balaban and Rasooly, 2000**). *Staphylococcu*s produce some enzymes which are invasiveness heat stable entero toxines that renders the food dangerous even it appear normal and extensive cooking can kill the organism but the toxins cannot be destroyed and very dangerous for health **(Prescott *et al*., 2005**). Infections with *Salmonella* and *Staph. aureus* are the causative agents of two third (2/3) of food borne disease outbreaks due to contamination during processing of meat products (**Khan *et al.,* 2014**). On the other hand, Aflatoxins are the most important mycotoxins, produced by *Aspergillus flavus* and *Aspergillus parasiticus* strains and pose a quadruple threat to both human and animals as they produce four distinct effects: acute liver damage, liver cirrhosis, induction of tumors and teratogenic effects **(Wogan *et al*., 1974 and Pitt and Hocking, 1997**)**.** It is worth to mention that heat treatment as cooking and roasting aflatoxins in food unable to destroy **(Trucksess *et al.,* 1988).** Rapid detection methods can be categorized into nucleic acid-based, biosensor-based and immunological-based methods **(Law *et al.*, 2015)**. PCR (polymerase chain reaction) and in particular multiplex PCR (mPCR) were proven as one of the most suitable way of approaching the issue of bacteria detection **(Sun *et al.*, 2011; Brizzio *et al.*, 2013; Hummerjohann *et al.*, 2014; Cremonesi *et al.*, 2015; Zeinhom *et al.,* 2015 and Shawish *et al.*, 2016).**

In this study, the focus is on the occurrence of various types of moulds with special reference to aflatoxigenic species, their screening for aflatoxin producing ability and aflatoxin residues in meat and meat products associated with risks to humans. Therefore, the present work was undertaken to evaluate both the traditional and molecular biology methods for detection and characterization of the aflatoxigenic Aspergillus and bacterial isolates that recovered from meat product.

Materials and Methods

1- Collection of Samples:

A total of **80** random samples of some meat products as, Minced meat, Sausage, Basterma and luncheon (**20 of each**), were collected under aseptic condition from different groceries and supermarkets in Giza and Cairo governorates in a sterile polyethylene bags and sent to laboratory in ice box as soon as possible**.**

2– Bacteriological examination:

Preparation of Samples: according to **IC-MSF (1978)** 25 gm of each samples using a sterile scalped and forceps were transfer to Stomacher bag contain 225ml. of 0.1% peptone water and pummeled in a stomacher (**Seward Stomacher BA 7021. England)** for 2 min. to obtain a dilution of 1/100, then decimal dilution were prepared using the same dilution.

***Aerobic plate count (APC):** According to **FAO (1992).**

3-Mycological analysis

Preparation of samples:

According to (APHA, 2003), the prepared samples are cultured Saboraud dextrose agar medium and incubated at 25°C for 3-5 days. The counts of mould colonies were recorded. Individual suspected colonies were selected depending upon their morphological characters. Stock culture were made from each isolate and monitored on Czapeks Dox, malt extract and potato dextrose (PDA) agar slopes for further identification.

Identification of isolates of moulds:

According to (**Raper and Fennel (1965)** and **(Pitt JI and Hocking AD (2009).**

Identification of isolates of bacteria:

Isolation and Identification of *Salmonella*: according to (**ISO- 6579 /2017)**:

Pre-enrichment media: buffered peptone water (OXOID-UK) and incubated at 37 c for 24h

Enrichment media:

-Rappaport-Vassiliadis (RV) with soya broth (OXOID-UK) then incubated at 41.5 c for 24h

-Muller-Kauffmann Tetrathionate with novobiocine broth (MKTT) then incubate at 37 c for 24h.

Plating specific media

-**XLD** and **S.S** agar, incubated at 37^{O}C for 24h., suspected colonies were examined for:

Biochemical identification**: (**Quinn *et al.,

2002), Using the following tests were (Indol, MR, VP, S.Cit, Urea, Lysine decarboxylation, TSI, Sugar fermentation, Oxidase and Catalase).

***Serological identification:** (**ISO-6579/2014**)

Isolation and Identification of *Escherichia coli*: according to (**European Pharmacopoeia, 2007)** was determined by inoculation of samples on to Eoisin Methylene Blue and MacConkey agar then incubated at 30°C-35°C for 18-24hrs.

***Biochemical reactions**: according to (**Quinn *et al*., 2002**), the following tests were (Indol, MR, VP, S.Cit, Urea, Lysine decarboxylation, TSI, Sugar fermentation, Oxidase and Catalase).

***Serological Identification**: according to (**Neville and Bryant, 1986**), *Escherichia coli* antisera were obtained from (Denka Seiken Co., LTD. Tokyo, Japan) used for serological identification of *E. coli*.

Isolation and Identification of *E. coli O157: According to **Doyle *et al.* (1997) and ISO 16654 (2001).**

Isolation and Identification of *Staph. aureus: According to (**FDA, 2001**). The prepared samples were streaked on the surface of Baird Parker agar (OXOID-UK) plates. Then incubated at 37°C for 24-48hrs, suspected colonies were picked up in to slant of nutrient agar for further identification by biochemical reactions and Coagulase activity according to (**Quinn *et al.*, 2002**).

Extraction of aflatoxin:

Extraction of aflatoxin from *A. flavus* and *A. parasiticus* isolates according to **(Gabal *et al.*, 1994 and AOAC, 1995).**

A. flavus and *A. parasiticus* isolated from present samples were inoculated into flasks containing 50 ml of sterile yeast extract solution 2% and 20% sucrose (YES). Inoculated flasks were incubated at 25ºC for 15 days. At the end of the incubation period, the flask content was filtered to separate the mycelial mat from YES medium. At the end of incubation period, extraction and detection of aflatoxin was estimated by TLC method as recommended**.**

Aflatoxins standard solution for TLC:

AFs standard B1, B2, G1, and G2 were purchased from (Sigma Chemical Company, St. Louis U.S.A).

Detection of aflatoxigenic *A. flavus* by polymerase chain reaction (Bintvihok *et al*., 2016)

DNA extraction. DNA extraction from samples was performed using QIAamp DNeasy Plant Mini kit (Qiagen, Germany, GmbH). Briefly, 100 mg of the sample was added to 400 µl Buffer AP1 and 4 µl RNase A stock solution (100 mg/ml), tungsten carbide bead were added to the previous mixture in a 2 ml safelock tube. Tubes were placed into the adaptor sets, which are fixed into the clamps of the Tissue Lyser. Disruption was performed in two 1–2 minute high-speed (20–30 Hz) shaking steps. The mixture was incubated for 10 min at 65°C and mixed 2 or 3 times during incubation by inverting tube. Then, 130 µl Buffer P3 was added to the lysate, mixed, and incubated for 5 min on ice. lysate was centrifuged for 5 min at 14,000 rpm., and then pipetted into the QI A shredder Mini spin column (lilac) placed in a 2 ml collection tube, and centrifuged for 2 min at 14,000 rpm. The flow-through fraction was transferred into a new tube without disturbing the cell-debris pellet and then applied to silica column. The lysate was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 50 µl of elution buffer provided in the kit.

B. Oligonucleotide Primers. Primers used were supplied from Biobasic Canada and are listed in table (1).

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions for conventional PCR.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Aflatoxin B1 aflR	AAC CGC ATC CAC AAT CTC AT AGT GCA GTT CGC TCA GAA CA	800	94˚C 5 min.	94˚C 30 sec.	50˚C 1.25 min.	72˚C 1.40 min.	72˚C 10 min.	**Bintvihok, *et al.*, (2016)**

Table (B). Primers sequences, target genes, amplicon sizes and cycling conditions for conventional PCR.

Target Bacteria	Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
Staph	*Hlg*	GCCAATCCGTTATTAG AAAATGC CCATAGACGTAG-CAACGGAT	937	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	**Kumar *et al.*, (2009)**
	icaD	AAA CGTAAG AGA GGT GG GGC AAT ATG ATC AAGATA	381	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Ciftci *et al.*, (2009)**
E. coli	*iss*	ATGTTATTTTCTGCCG CTCTG CTATTGTGAGCAA-TATACCC	266	94˚C 5 min.	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Yaguchi *et al.*, (2007)**
	iroN	ATC CTC TGG TCG CTA ACT G CTG CAC TGG AAG AAC TGT TCT	847	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	**Ewers *et al.*, (2007)**
Salmonella	avrA	CCT GTA TTG TTG AGC GTC TGG AGA AGA GCT TCG TTG AAT GTC C	422	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Huehn *et al.* (2010)**
	sop B	tca gaa gRc gtc taa cca ctc	517	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	

C. PCR amplification.

DNA samples: Primers were utilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

D. Analysis of the PCR Products.

Conventional PCR products: The products of PCR were separated by electrophoresis on1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A gelpilot 100 bp DNA Ladder (Qiagen, Germany, GmbH)and generuler 100 bp DNA ladder (Fermentas, Thermo) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Statistical analysis

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

Results and Discussion

Meat and meat products are considered as a major vehicle of most reported food poisoning outbreaks, therefore it is important to use the microbiological criteria as it gives guidance on the acceptability of meat products and their manufacturing, handling and distribution processes (**Hassanien, 2004**). The consumption of food contaminated with moulds and their toxic metabolites results in the development of food-borne mycotoxicosis**.** Advanced countries considered the mould counts as a standard test for hygienic condition due its economic and public health effects **(Ismail *et al.*, 1995)**

Table (1). Statistical analytical results of Total Mould Counts in meat product samples (n=20):

Examined samples	No. of examined samples	Positive samples		Colony Count Count (cfu)/g		
		No.	%	Min.	Max.	Average
Luncheon	20	16	80	1x10	20 $x10^3$	4.8 $x10^3$
Basterma	20	12	60%	$10x10^2$	11 $x10^3$	4.7 $x10^3$
Sausage	20	10	50%	1 x10	1 $x10^3$	4.1 $x10^2$
Minced meat	20	12	60%	$1x10^2$	$2x10^3$	$6.1x10^2$

As revealed in Table (1) it was noticed that the highest average of total mould count/g was obtained from the luncheon samples (4.8 $x10^3$) followed by basterma samples (4.7 $x10^3$) and minced meat ($6.1x10^2$); whereas the sausage samples (4.1 $x10^2$) yielded the lowest count of mould. These results come in accordance with those reported by **Nouman *et al.*, (2001)** and **El-Tabiy (2006).** The high incidence of moulds in sausage and other meat products may be due to the frequent unhygienic handling and processing of meat especially when additives of low quality as flavorings, especially, spices were used **(Wafia and Hassan, 2000)**. Egyption Organization for Standardization and Quality Control **(EOSQC, 2005),** recommended that the total colony count of mold and yeast not exceed than 10^2 cfu/g in Basterma, while, there is no primissible limit for launchon and Susage.

Table (2). Incidence of mold isolated from the examined meat product (n=20 of each):

Molds spp. \ Products	Luncheon		Basterma		Sausage		Minced meat	
	No.	%	No.	%	No.	%	No.	%
Aspergillus species	16	80	11	55%	8	40%	10	50%
Aspergillus flavus.	10	50%	6	30%	4	20%	6	30%
Aspergillus parasiticus	4	20%	0	0%	1	5%	0	0
Aspergillus niger	2	10%	5	25%	3	15%	4	20%
cladosporium sp.	0	0	0	0	0	0%	1	5%
Pencellium species	0	0	1	5%	2	10%	1	5%

Table (2), it is evident that 3 genera were isolated and the most frequently encountered mould genera from the examined samples were *Aspergillus* with the average incidence of (40-80%) and while, *Penicillium* were 5-10% and *Cladosporium* species 0-5% were recovered at low percentages. Frequency of identified *Aspergillus* species isolated from examined meat products *Aspergillus* species were further identified. The most prevalent identified species were *A. flavus, A. parasiticus and A. niger*. Under the same conditions, other members of *Aspergillus* were recovered in variable frequencies. Nearly similar results were obtained by **El- Tabiy, (2006)**, **Ismail and Zaki (1999), Brr *et al.,* (2004) and Hassan, (2013).**
Aspergillus was known to be common contaminants of human foods and animal feeds (**Bullerman, 1979**). The presence of Aspergillus is not only of economic important but also represents a real health hazard. They can be of allergic, toxigenic and pathogenic effect through the production of mycotoxins **(Aldoory, 1980).**

Table (3). Aflatoxins production by different strains of *A. flavus* and *A. parasiticus* isolated from meat product samples:

Source of isolation	Total No. of isolates	Aflatoxins +ve strains		Levels of AFs (PPb)		
		No.	%	Min.	Max.	Mean± S.E.
Luncheon	14	7	50%	0.4	4.0	2.08± 0.54
Basterma	6	4	66%	0.8	8.0	4 ± 1.6
Sausage	5	3	60%	0.8	2.0	1.36± 0.35
Minced meat	8	6	75%	0.8	8.0	3.35± 1.09

Data presented in table (**3**) illustrated the aflatoxins production by isolated *A. flavus* and *A. parasiticus* strains from meat products samples. The highest incidence of aflatoxins was observed in the strain isolated from minced meat (75%), followed by Basterma (66%), Susasage (60%) and Launchon (50%). The highest mean values of aflatoxins residues were detected in basterma (**4±1.6** ppb), minced meat (**3.35±1.09** ppb) followed by luncheon (**2.08± 0.54** ppb) and the lowest level was detected in sausage (**1.36± 0.35** ppb), respectively.
This result is in consistent with those obtained by **Rodrigues *et al.,* (2009)** and **Jamali *et al*., (2013).** These results were similar by **Brr *et al.,* (2004)** found the highest average quantity of aflatoxins was in sausage and beef burgers samples. These findings agreed with hat recorded by **Aziz and Youssef (1991);** while **El-Gazzar, (1995**) recorded extreme higher incidence attained to 100%. A relatively lower incidence were obtained by **El- Shawaf, (1990)**.
On the other hand, the food borne diseases caused mainly by *Salmonella spp*., *Escherichia coli* and *S. aureus* are the major causes of mortality and infections specially in the developing countries. This bacteria are transmitted mainly through consumption of contaminated food and the presence of those organisms in meat and meat products have relevant public health implications (**Normanno, 2007**) and (**Zafar *et al.,* 2016**).

Table (4). The incidence of bacteria isolated from meat products:

Bacterial species	Prevalence of bacterial species in meat products									
	Minced meat		Sausage		Basterma		Luncheon		mean±SEI	
Total bacterial count	$2.5x10^3$±2.3		$1.5x10^3$±1.6		$2.1x10^3$±1.5		$4.1x10^2$±3.0		$2.5x10^3$±2.1	
Incidence of isolates	No.	%	No.	%	No.	%	No.	%	No.	%
Escherichia coli	3	15%	3	15%	4	20%	2	10%	12	15%
Salmonella spp	2	10%	2	10%	1	5%	-	-	5	6.25%
Staph. aureus	5	25%	4	20%	4	20%	2	10%	15	18.75%

According to the results in **table (4)** the mean of total bacterial count in minced meat, sausage, Basterm**a** and in luncheon were **(**$2.5x10^3$±2.3, $1.5x10^3$±1.6, $2.1x10^3$±1.5, $4.1x10^2$±3.0). The results of **APC** are nearly similar to those recorded by **Hamed *et al* (2015)** and **Ahmed (2015).**

Egyptian Organization for Standardization and Quality Control **(EOSQC, 2005)**, recommended that the APC not exceed than 10^4 cfu/g and there is no primissible limit for Enterobacteriaceae count while Coliform counts must be not more than 10^2 cfu/g and *Staphylococcuus aureus* counts must be =0 cfu/g.

Table (4) showed that, the bacterial isolates from samples of meat products were identified as *E. coli* (15%), *Salmonella* sp. (6.25%) and *Staph. aureus* (18.75%) this results nearly similar to **Amany *et al.,* (2015**) who recorded that *E. coli* (14.2%), *Salmonella* sp. (3%) but high percentage of *Staph*. *aureus* (28.57%) While this results are differs than that recorded by **Hassanein *et al.,* (2014)** and **Khanam *et al*., (2013)**, who isolated high incidence of *Salmonella* (**20%**) and *E. coli* (45%).

The presence of these organisms in meat products may be as a result of poor hygiene and sanitary practice employed in the slaughtering, processing and packing of meat products. **Michael *et al.,* (1981**) and **Amany *et al.,* (2015).**

Staph. aureus isolated from all meat products samples were 15 (18.75%) with incidence of 25%, 20%, 25% and 10% from minced meat, sausage, basterma and luncheon respectively. *Staph. aureus* was responsible for 25% of all food borne outbreaks in USA, while in UK, the meat products of animal origin constitute75% of outbreaks incidence caused by *Staphylococca*l food poisoning (**Balaban and Rasoly, 2000).**

A variable percentage were reported by **Torky (2004**) who found that the incidence of *Staph. aureus* were 20%, 15%, 35%, 5%, and 25% in Beef burger, Luncheon, Minced meat, Basterma and sausage respectively.

Table (5). Serotypes of *Salmonella* isolated from samples of meat *product*

Types of meat products	Serotypes Salmonella									
	Salmonella Typhimurium		***Salmonella Enteritidis***		***Salmonella Agama***		***Salmonella Paratyphi A***		**Total**	
Meat products	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Minced M.	1	5%	-	-	1	5%	-	-	2	10%
Sausage	1	5%	1	5%	-	-	-	-	2	10%
Basterma	-	-	-	-	-	-	1	5%	1	5%
Luncheon	-	-	-	-	-	-	-	-	-	-
Total	2	2.5%	1	1.25%	1	1.25%	1	1.25%	5	6.25%

In this study, **Table (**5) recorded that, the incidence of ***Salmonella*** was (**6.25%**) of all examined meat products, this results nearly agree with the findings reported by **Cortez *et al.* (2008**) who reported the same results of salmonella (7.5%) also **Siriken *et al.* (2006)** who obtained 7% of *Salmonella*. Our results high than the obtained by (**Amany *et al.,* 2015**) who obtained the incidence of *Salmonella* with 3% and **Abd EL-Twab *et al. (*2013**) who found incidence of *Salmonella* was (1.33%). While, the our data were lower than the results obtained by **Mrema *et al.* (2004**) who recorded high incidence of *Salmonella* (20%).

In the same table, it is clear that 4 strains of isolated *Salmonellae* from meat products *(S. yphimurium, S. enteritidis, S. agama* and *S. paratyphi* A) were 6.25% from all examined meat products, *S*. Typhimurium (two strains) was an incidence of 2.5% of all Salmonellae isolates. These results agree with that reported by **Ahmed and Shimamoto (2014)** who isolate *Salmonella* Typhimiurium (2.5%) while, our results are lower than data were recorded by **Esam (2010)** who isolate with an incidence 8%. While *Salmonella enteritidis* (one strain) with an incidence of 1.25%, this results lower with the other previous studies (**Molla *et al.,* (2003)** and **Charles Hemades *et al.* (2006**) with an incidence of 12.1% and 20% respectively.

One strain of each of *Salmonella* Agama from minced meat and *Salmonella Paratyphi* A isolated only from Basterm) these results were agree with that reported by **Nouman *et al.* (2001**) who found *S. paratyphi* A in Basterm samples, this may be due to poor hygienic measures and low quality controls or may be from hands of workers of the processing line of production. On other hand *Salmonellae* failed to be isolated from luncheon samples this results agreed with **EL-Dosoky *et al.* (2003)**. Absence of *Salmonella* isolated in luncheon samples may be the addition of food additives such as spices and preservatives which have an antimicrobial activity and inhibit survival and multiplication of Microorganisms (**libby, 1975)**. This also may be attributed to the exposure to high temperature during processing and cooking procedures (**Hoesin *et al.,* 2008**). The prevalence of *Salmonella* in meat products may indicate the contamination of meat products during processing as well as contamination from grinding, air, packing materials and hands of the workers, also temperature rise (2^{o}C-4ºC) during grinding could also increase the contamination of *Salmonella* (**Ismail, 2006).**

Table (6). Incidence of identified of *E. coli* and *Staph. aureus* isolated from examined meat products

Meat products	Minced meat		Sausage		Basterma		Luncheon		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
1- *E. coli* *E. coli O20*	1	5%	-	-	1	%5	-	-	2	10%
E. coli O26	1	5%	1	5%	1	5%	-	-	3	15%
E. coli O86	-	-	-	-	1	5%	-	-	1	5%
E. coli O111	1	5%	1	5%	2	10%	1	5%	5	25%
E. coli O157	-	-	-	-	1	5%	--	-	1	5%
Total	3	3.75%	2	2.5%	6	7.5%	1	1.25%	12	15%
2- *S. aureus:* A. Coagulase (+ve)	13	65%	12	60%	14	70%	11	55%	50	62.5%

From **table (6)** it is clear that the *E. coli* isolated from the all examined samples of meat products with percentage of 15%. This results nearly agree with that obtained by **Amany *et al.*, (2015)** who isolated *E. coli* from meat products with an incidence of 14.2%, also this **table (6)** showed that identified serotypes of *E. coli* (12) were (2) strains of O20 isolated with 5% from Minced meat and Basterma, (3) strain of *E. coli* O26 from minced meat, Sausage and Basterm (5% of each). While, one strain of each (*E. coli* O86 and *E. coli* O157) only could be isolated from Basterma samples with (5%) and 5 strain of *E. coli* O111 isolated from all samples of meat products. This results nearly agree with **Ebtesam *et al.*, (2014**) who reported that ETEC (O20 & O26) & EHEC (O111 & O157) and EPEC (O86) were isolated from samples of meat products.

Table (6) showed the incidence of identified *S. aureus* isolated from examined meat products samples (minced meat, sausage, basterma and luncheon) the positive coagulase *S. aureus* of the examined meat products samples were 65%, 60%, 70% and 55% respectively. The incidence of *S. aureus* coagulase positive was 62.5% these results nearly agree with that results obtained by **Amany *et al.*, (2015**) who recorded *S. aureus* coagulase positive (70%). The highest incidence of *S. aureus* may be due to very bad hygienic measures in many supermarkets **(Hayes, 1992**).
Food contaminated by *Staphylococcus aureus* may occur directly from infected food producing animals or may result from poor hygiene during production, processing and storage.

Recently, rapid and more objective methods for the identification of mycotoxigenic fungi in human foods and animal feeds are needed for evaluating the microbiological risks of a given food products. So, molecular biology techniques are increasingly used in the identification of fungi and yeasts **(Abo El Yazeed *et al.*, 2011).** In general, the accuracy of the PCR was found to be 70-85%, where PCR proved to be a rapid diagnostic technique for detection of pan fungal genome directly from clinical specimens **(El Hamaky *et al*., 2016)**. The sensitive and specific nested PCR assay as well as the rapid and quantitative Light-Cycler PCR assay might be useful for the diagnosis and monitoring of fungal infections **(Bialek *et al*., 2002 and Abo El Yazeed *et al*., 2011)**.

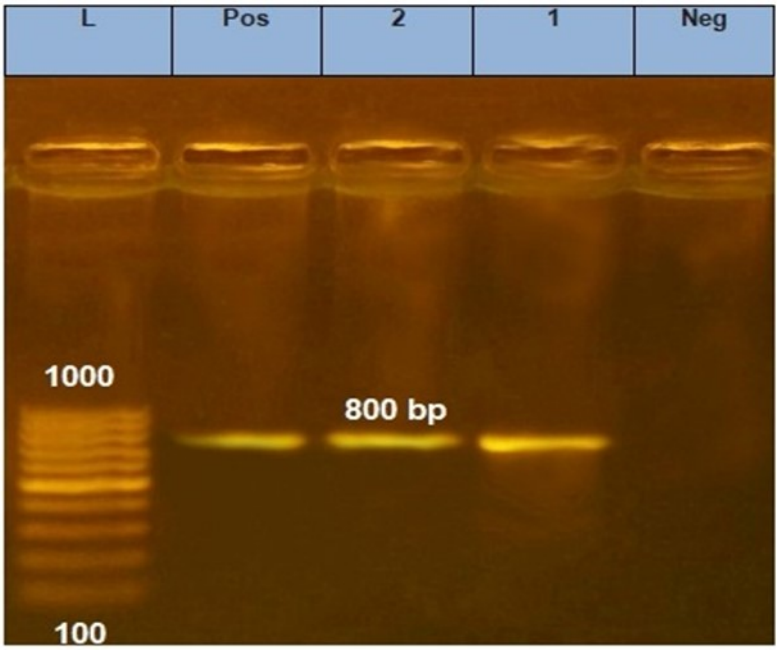

Figure (1). PCR detection using Afla R primers which is specific for *A. flavus.*
Lan L:100pb PCR markers. Lan pos.: control positive. Lan2, 1: *A.flavus* strains, Lan neg: negative control

In the present study the detection of toxigenic and non-toxigenic *A. flavus* by PCR produced similar pattern during sequencing using *alfa R* primers and no differences detected between both isolates.

PCR using Afla R primers responsible for aflatoxin production identify toxigenic *A. flavus* at (800 bp) for the two examined strain.

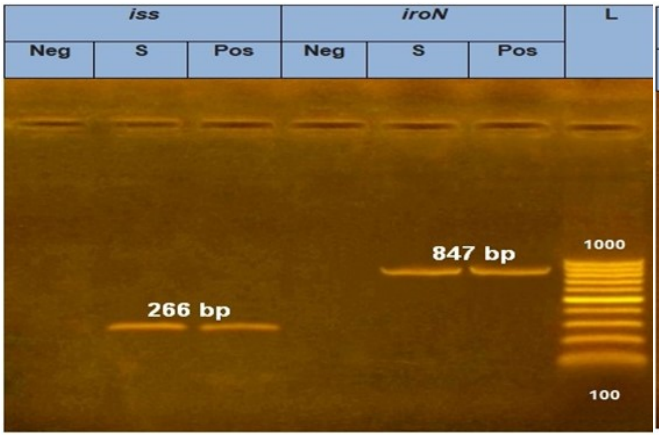

Figure (2). PCR detection of ***E. coli genes***
Iss gene (Lan. Neg: negative control, Lan S: the isolates, Lan. Pos: Positive control).
IroN gene (Lan Neg: negative control, Lan S: the isolate, Lan Pos: Positive Control).
Lan L: 100 pb PCR markers.

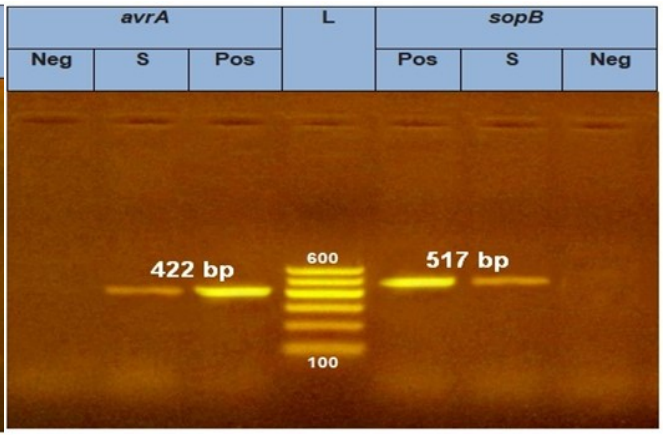

Figure (3). PCR detection of ***salmonella genes***
Avr A gene (Lan. Neg: negative control, Lan S: the isolates, Lan. Pos: Positive control).
Lan L: 100pb PCR markers.
sopB gene (Lan pos: Positive control, Lan S: the isolate, Lan. Neg: negative Control).

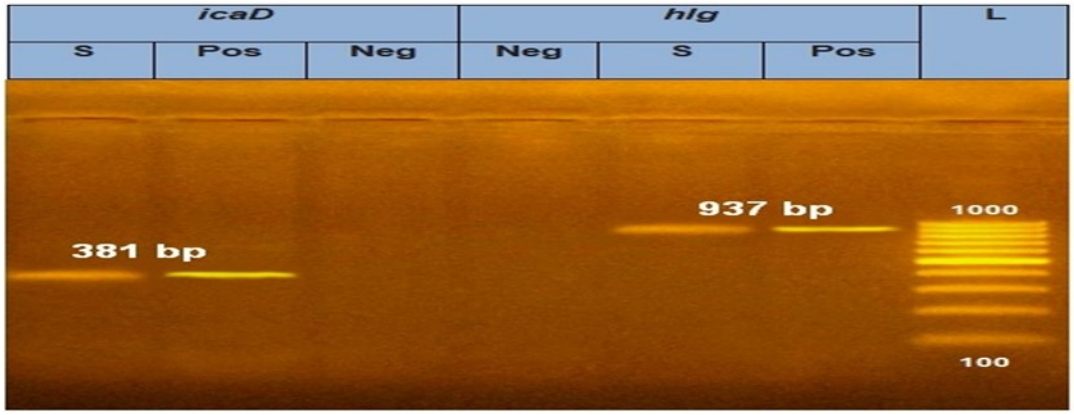

Figure (4). PCR detection of ***Staph. aureus***
icaD gene (Lan S: the isolates, Lan Pos: Positive Control, Lan Neg: negative control)
hlg gene (Lan Neg: negative control, Lan S: the isolate, Lan Pos: Positive Control)
Lan L: 100pb PCR markers. .

E. coli detection in foods is one of the most useful hygienic criteria **(Scheinberg *et al.*, 2017 and Simancas *et al.*, 2016).** However, at present the conventional method for *E. coli* detection requires several days **(Stromberg *et al.*, 2015),** especially in cases where *E. coli* concentrations are low. Enrichment is a commonly used method for bacterial isolation to increase the cell counts of target bacteria above other background flora prior to identification **(Gracias and McKillip, 2004).**

In the present study, the PCR using primers against (iss and iroN genes) were used to identify *E. coli* at (266 bp and 847 pb), respectively (figure, 4). Similar results were detected by **(Molina *et al.*, 2015)** who used PCR, using primers against the *uidA* gene that encodes beta-D-glucuronidase to identify *E. coli* accurately.

In the present study, the *Salmonella* target genes (avr A and Sop B) were detected at (422 pb and 517 pb), respectively (Fig. 3). This showed a high accuracy in detecting *Salmonella* from pure culture as well as in regulatory food samples **(Cheng *et al.* 2005).**

In addition, the detection of genes for enterotoxin (icaD and hIg) from *Staph. aureus* has also detected by PCR at (381 bp and 937 bp) , respectively (Figure, 4). Similar findings were reported by **Johnson *et al.* (1991).**

Conclusion

The high incidence of bacteria and fungi in meat products warrants that there is urgent need to apply strict hygienic measures during the processing of meat products. Low microbiological quality is associated with storage above 8ºC, infrequent cleaning of the equipment and poor control practice that likely lead to cross contamination. It has further evidenced that the undesirable level of contamination which might have acquired from the environment and agents and to obtain wholesome, safe and sound meat products, the principles Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Point (HACCP) must be adopted.

Application of molecular biology technique was found to be rapid, highly specific, easy to perform and effective method to assist creation of such programs and reduction of the risk of harmful effects of food-poisoning bacteria and toxigenic fungi and their toxins to human and other farm animals health.

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