Assiut University web-site: www.aun.edu.eg

PREVALENCE OF VIRULENCE GENES OF SOME FOODBORNE BACTERIA IN CHICKEN MEAT PRODUCTS

NASHWA, M. ZAKI [1]; EL-DOSOKY. H.F.A[1]; and WAFAA, M. GAD[2]
[1] Food Hygiene Dept. Mansoura Provential Lab, Animal Health Research Institute, Egypt
[2] Microbiololgy Dept. Mansoura Provential Lab, Animal Health Research Institute, Egypt

Received: 23 March 2017; **Accepted:** 13 April 2017

ABSTRACT

This Study was carried out on 200 random samples of chicken meat products represented by chicken luncheon, chicken burger, chicken sausage and chicken shawerma (50 of each). Samples were randomly collected from different supermarkets and retailers of different sanitation levels at Mansoura city, El Dakahlia Province, Egypt and bacteriologically analyzed to assess the prevalence of *Staph. aureus*, *E. coli* and *S.* spp. and their enterotoxigenic virulence genes using PCR in some chicken meat products intended for direct consumption. The obtained results revealed that the prevalence of *Staph. aureus in examined* chicken luncheon, chicken burger, chicken sausage and chicken shawerma were 6%, 2%, 2% and 2%., respectively. While *E. coli* were 2%, 4%, 0% and 2% in examined samples respectively and *S. spp.* was isolated by 2% from shawerma only. The isolated *S. typhimurium* harbor *inv*A and *stn* genes. The isolated *E. coli* showed presence of shiga toxin genes (*stx*1 and *stx*2). The examined coagulase positive *Staph. aureus* showed the presence of different enterotoxin genes *sea*, *seb*, *sec*, *sed* and *see*. Thus it is necessary to adopt a regime of good, safe and healthy production of such chicken meat products with cleaning and disinfection and hygienic packaging in order to ensure safe products for consumers.

Key words: *Prevalence, Virulent Genes, Foodborne Bacteria, Chicken, Meat Products.*

INTRODUCTION

Poultry meat and its products are very popular food throughout the world, it considered as cheap, good delicious and nutritious, source of protein with good flavour and easily digestion. Ready to eat food can be described as the status of food being ready for immediate consumption at the point of sale, it may be raw or cooked, and can be consumed without further treatment Tsang (2002). The importance of food as a vehicle for transmission of several diseases has been documented, especially in developing countries where the hygienic standards are not strictly followed or enforced Harakeh *et al.* (2005). *Staph. aureus* produces a wide variety of toxins including staphylococcal enterotoxins (SEs; SEA to SEE, SEG to SEI, SER to SET) with demonstrated emetic activity, SEs are a major cause of food poisoning, which typically occurs after ingestion of different foods, particularly chicken meat products, contaminated with *Staph. aureus* by improper handling and subsequent storage at elevated temperatures Tharwat and Elabbasy (2014). Salmonellosis was one of the most commonly zoonotic disease accounting for 133,258 confirmed humancases Osek and Wieczorek (2010). *Salmonella* often present in fresh tissues due to defects during slaughtering process of poultry and carcass manipulation Lee *et al.* (1998) as well as Cebedo *et al.* (2008) concluded that S. spp. are pathogenic bacteria that can contaminate food products during or after processing. Hamilton *et al.* (2009) mentioned that *E.coli* was isolated from 60% of examined poultry from butcher shops with mean counts of 0.70 $\log_{10}$ cfu/g. and 16% from poultry sold in supermarket samples with mean counts of 0.51 $\log_{10}$ cfu/g. Matossian and Kingcott (1979) detected food poisoning outbreak from donar kebab (a product similar to shawerma). *Staphylococcus spp*., *E.coli* and *S. spp*. were isolated from raw chicken products and chicken shawerma Kaneko *et al*. (1999) and Pelczar *et al*., 2006). Shiga toxin (Stx)-producing *E. coli* (STX-EC), also known as Verotoxin-producing *E. coli* which associated with infantile diarrhea, haemorrhagic colitis, thrombocyticpurpura, and hemolytic uremic syndrome in humans Griffin and Tauxe (1991). The aim of this study was to assess the presence of these bacteria in some chicken meat products and the risk of contamination on the consumer.

Corresponding author: Dr. NASHWA, M. ZAKI
E-mail address: nashwazaki80@gmail.com
Present address: Food Hygiene Dept. Mansoura Provential Lab, Animal Health Research Institute, Egypt

MATERIALS AND METHODS

1- Collection of samples:
Two hundred samples of chilled chicken meat products (50 samples each of chicken luncheon, chicken burger, chicken sausage and chicken shawerma) at ± 4^{o} C were collected aseptically from different shops (small grocery and large supermarkets) from Mansoura city, Dakahlia province and transferred to the laboratory in an insulated ice-box without delay.

2- Bacteriological examination:

2.1- Preparation of food homogenate: according to technique recommended by ISO, 6887-2, (2003) 25 g. of each sample was removed by a sterile scissors and forceps and stomached using Seward stomacher 80 biomaster England with 225 ml sterile buffered peptone water (0.1%) to give a homogenate of 1/10 dilution from which ten fold serial dilutions were prepared and subjected to the following bacteriological examination.

2.2- Total *E.coli* count: according to technique recommended by FDA (2002a).

2.3- *Staphylococcus aureus* count: FDA (2002b). using Baird-Parker agar plates, incubated at 35 ^{o}C for 48 hr. The suspected *Staphaureus* colonies were isolated, purified and confirmed by coagulase test and the total count was calculated.

2.4- Isolation of *E. coli* according to technique recommended by ISO, 16649/2, (2001)

2.5- Isolation of *Salmonellae* ISO, 6579 (2002): by enrichment in peptone water at (37 ^{o}C for 24hr) then selection enrichment in Tetrathionate (37 ^{o}C for 24hr) and rappaportvasiliades at 41.5 ^{o}C for 18 hr., platting on XLD, MaCconkey's and Hektoneentreic agar at 37^{o}C for 24 hr. The presumptive colonies were confirmed biochemically and serologically.

3- Detection of virulence genes in *Staphaureus, E. coli* and *Salmonella* using PCR:
Carried out in Reference Lab for Quality Control on Poultry Production, Animal Health Research Institute, Dokki-Egypt.

3.1- DNA extraction:
DNA extraction from positive samples were(6 *Staph. aureus*), (4*E. coli*) and (*1 Salmonella*) performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The samplewas then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

3.2- Oligonucleotide Primers:
The used Primers used were supplied from Metabion (Germany) are listed in Table (I) and Table (II).

3.3- PCR amplification:
For uniplex PCR, primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. For stx1, stx2 duplex PCR, primers were utilized in a 50- µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 13 µl of water, and 8 µl of DNA template. The reaction was performed in an appliedbiosystem 2720.

3.4- Analysis of the PCR Products:
The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the uniplex PCR products and 30 µl of the duplex PCR products were loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Thermo Scientific, Germany) was 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.

Table I: primer sequence for *Staph. aureus* enterotoxins genes used in multiplex PCR (Mehrotra *et al.*, 2000).

Primer pairs	Nucleotide sequence(5′→3′)	Amplicon size (bp)
sea Forward Reverse	5` GGTTATCAATGTGCGGGTGG 3` 5` CGGCACTTTTTTCTCTTCGG 3`	102 bp
seb Forward Reverse	5` GTATGGTGGTGTAACTGAGC 3` 5` CCAAATAGTGACGAGTTAGG 3`	164 bp
sec Forward Reverse	5`AGATGAAGTAGTTGATGTGTATGG 3` 5` CACACTTTTAGAATCAACCG 3`	451 bp
sed Forward Reverse	5` CCAATAATAGGAGAAAATAAAAGG 3` 5` ATTGGTATTTTTTTTCGTTC 3`	278 bp
see Forward Reverse	5`AGGTTTTTTCACAGGTCATCC 3` 5`CTTTTTTTTCTTCGGTCAATC 3`	209bp

Table II: Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
stn	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	617	94˚C 5 min.	94˚C 30 sec.	59˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	**Murugkar *et al.*, 2003**
invA	GTGAAATTATCGC CACGTTCGGGCAA TCATCGCACCGTC AAAGGAACC	284	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min	**Oliveira *et al.*, 2003**
Stx1	ACACTGGATGATC TCAGTGG CTGAATCCCCCTC CATTATG	614	94˚C 5 min.	94˚C 30 sec.	58˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	**Dipineto *et al.*, 2006**
Stx2	CCATGACAACGGA CAGCAGTT CCTGTCAACTGAG CAGCACTTTG	779						

Statistical analysis:
The results are expressed as mean ± standard Error (SE). Data were statistically analyzed using statistical analysis systems. (SAS version 9.1, SAS Institute, Inc., 2003).

RESULTS

The achieved results of *Staph. aureus* in Tables (1 & 2) for Chicken luncheon, Chicken burger, Chicken sausage and Chicken shawerma were 3.2±1.6, 3.5±1.5, 3.6±1.4 and 3.7±1.3 $\log_{10}$cfu/g. with incidence rate 6%, 2%, 2% and 2 %, respectively. The results of *E. coli* in Tables (1,2) for Chicken luncheon, Chicken burger, Chicken sausage and Chicken shawerma were 3.4±1.8, 3.1±1.3, 3.7±2.1 and 3.8±1.5 $\log_{10}$ cfu/g. with incidence rate 2%, 4%, 0% and 2%, respectively, serologically the isolated *E. coli* indicates presence of the enterotoxigenic strains *E. coli* O127:H6 in chicken luncheon and *E. coli* O125:H21 and *E. coli* O127:H6 in chicken burger. *Salmonella* were not detected in Chicken luncheon, Chicken burger and Chicken sausage and detected in 2% of the examined chicken shawerma was *S. typhimurium*.

By PCR the results showed the presence of enterotoxin producing genes (A, C, D and E) in *Staph. Aureus* the three *isolates of Staph. aureus* isolated from luncheon showed the presence of enterotoxin gene 1st (A, E) , 2nd (A, D) and 3rd (B, D). The isolate of *Staph. aureus* isolated from burger showed presence of enterotoxin genes (A and E), the sausage isolate showed the presence of enterotoxin gene (A) while shawerma isolate showed presence of enterotoxin genes (A) .The virulence genes of shiga toxin (*stx*1 and *stx*2) were examined using PCR in the four *E. coli* isolates the results were postive for these genes.

Table1: Statistical analytical results of *Staph. Aureus* and *E. coli* in the examined samples expressed as log_{10}cfu/g.(n=50).

Microbial count log_{10}cfu/gm ±S.E.	Chicken luncheon	Chicken burger	Chicken sausage	Chicken shawerma
STAPH.aureus	3.2±1.6	3.5±1.5	3.6±1.4	3.7±1.3
E. coli	3.4±1.8	3.1±1.3	3.7±2.1	3.8±1.5

Table 2: The incidence, Serotyping and virulence gene of isolated, *Staph. Aureus, E. coli* and S. spp. from the examined samples (N= 50 of each).

samples	Chicken luncheon		Chicken burger		Chicken sausage		Chicken shawerma	
Strains	NO %	Strains & virulence gene	NO %	Strains & virulence gene	NO %	Strains & virulence gene	NO %	Strains & virulence gene
Staph. aureus	Cp 3(6%)	1st *sea, see* 2nd *sea, sed* 3rd *seb, sed*	Cp 1(2%)	*sea, sed*	Cp 1(2%)	*sea*	Cp 1(2%)	*sea*
E. coli	1 (2%)	ETEC O127:H6 *Stx2*	2 (4%)	1st ETEC O125:H21 *Stx1 and stx2*	ND -	-	1 (2%)	ETEC O125:H21 *Stx1 and stx2*
S. SPP.	ND	-	ND	-	ND	-	1 (2%)	*S. typhimurium inv* A, and *stn,*

ND. = not determined
No. = number of positive samples, C p =coagulase positive

DISCUSSION

Staph. aureus, E. coli and *Salmonella* are the major causes of food borne infection and intoxication and their presence in food conistitute an important hygienic problem for food processors, food handlers and consumers Bergadol (1989). The enterotoxication generally is not lethal and the elderly are more susceptible than the younger individuals, where the amount of *STAPH. aureus* enterotoxins required for intoxication about 94-184 ug Erol and Iseri, (2004).

The achieved results of *Staph. aureus* in Tables (1 & 2) for Chicken luncheon, Chicken burger, Chicken sausage and Chicken shawerma were 3.2±1.6, 3.5±1.5, 3.6±1.4 and 3.7±1.3 log_{10}cfu/g. with incidence rate 6%, 2%, 2% and 2 % respectively. The results nearly similar Saleh *et al.* (2010) *who mentioned that Staph. Aureus* count were $1.14x10^3$±$3.32x10^2$, $2.17x10^3$±$4.31x10^2$ and $2.2x10^3$± $4.45x10^2$/g. with different incidence of 4%,12% and 16% for luncheon, beef-burger and sausage respectively higher percentage were reported by Amal, (2004) 15% and 25% *in Staph. aureus* for luncheon and fresh sausage; Fatin, (2004) could isolate *Staph. Aureus* from luncheon in percentage of 16%; and Soultos *et al.* (2003) in percentage of 19.4% in luncheon; Mousa, (1993) reported that *S. aureus* count was $2.3x10^4$cfu/g. for luncheon.; Ahmed, (1992) 6.6% in sausage. EL-Mossalami *et al.* (2009) detected *Staph. aureus* in 92%, 80% and 88% with mean values of $3.25±6x10^3$, $2.8±1.4x10^2$ and $4.1±2x10^3$cfu/g. in sausage, beef burger and shawerma respectively; Armany *et al.* (2016) could isolate *S. aureus* in percentage of24% and 20% in raw sausage and luncheon; Shawish and AL-Humam (2016) were 12%,22% and 30% in beef luncheon, beef burger and beef sausage; AL-Ghamdi, (2012) *S. aureus* count in chicken luncheon and chicken burger were $1.47x10^6$ and $1.2x10^7$cfu/g respectively. Ibrahim, (2009) detected *Staph. aureus* in 22.85% and 31.85% in luncheon, and sausage and EL-Khatieb (1997) (29%) in sausage. the percentage of coagulase positive *Staph.* aureus strains isolated from Chicken luncheon, Chicken burger, Chicken sausage and Chicken shawerma were 6%,2%, 2% and 2% respectively as in Table (2). Chomvarin *et al.* (2006); Oh, *et al.* (2007) and Chiang *et al.* (2008) concluded that the occurrence of enterotoxigenic *Staph. Raureus* in ready to eat food products has been reported in various parts all over the world; Shalaby and Zaki, (2008) could isolate 4, 5 and 3enterotoxigenic strains of *Staph. aureus* from beef burger, sausage and shawerma respectively and Motten *et al.* (2011) found Coagulase positive *Staph.* aureus in luncheon by 7%, 7% and 5% from the collected samples from three supermarkets. Eldaly *et al.* (2014) showed that the isolation percentages of *Staph. aureus* in the

examined samples of luncheon, burger, and sausage were 15%, 10%, and 20% respectively.

The results of *E. coli* in Tables (1,2) for Chicken luncheon, Chicken burger, Chicken sausage and Chicken shawerma were 3.4±1.8, 3.1±1.3, 3.7±2.1 and 3.8±1.5 log_{10} cfu/g. with incidence rate 2%,4%,0% and 2% respectively, serologically the isolated *E. coli* indicates presence of the enterotoxigenic strains *E. coli* O127:H6 in chicken luncheon and *E. coli* O125:H21 and *E. coli* O127:H6 in chicken burger. These results nearly similar to Samaha *et al.* (2012) were 8% in chicken luncheon; Ibrahim (2009) were 5.71% in luncheon; Fawzy (2004) were 8% in luncheon and Amal (2004) were 5 and 25% in luncheon and fresh sausage. Meanwhile, higher results were recorded by Armany *et al.* (2016) 20% and 24% in raw sausage and luncheon respectively and Mousa (1993) were14% in luncheon; Ibrahim, (2009) were 42.85% in sausage and Fathi *et al*. (1992) in luncheon and sausage which were 41.67% and 20%which may be due to post processing contamination or unefficient cooking and improper handling.

Salmonella were not detected in Chicken luncheon, Chicken burger and Chicken sausage and detected in 2% of the examined chicken shawerma was *S. typhimurium*. EL Jakee *et al.* (2014) detect *Salmonella* in burger, sausage and poultry products by 10, 35 and 25% respectively, the isolated *Salmonella* were *S. enteritidis* and *S. typhimurium* and Samaha *et al.* (2012) could isolate 8% *Salmonella* in chicken luncheon, Amal (2004); Ibrahim (2009) and Saleh *et al.* (2010) can not find *Salmonella* in luncheon while in sausage Mousa (1993); Saleh *et al.* (2010)*;* Kozacinski *et al.* (2008); Ibrahim (2009); and Tudor *et al.* (2010) can't found *S. spp*. in fermented sausage. Amal (2004) found *salmonella* by 5% in sausage. The health hazard from *Salmonella* must not be underestimated. The fact that *Salmonella* was detected in samples from supermarkets, where chicken are displayed under refrigeration, shows that the spread of infection was not only confined to seemingly unhygienic environments FAO, (2013). It was suggested that to prevent contamination by *Salmonella* control measures must be taken at all stages of the food chain, from agricultural production, to processing, manufacturing and preparation of foods in both commercial establishments and at home WHO (2013).

PCR was applied to evaluate the presence of virulence genes in the isolated *Staph. aureus, E. coli* and *Salmonella*. *Staph. aureus* is one causes of food poisoning, its pathogenicity result from possession of virulence genes that produce different toxins which result in self-limiting sever illness. For this reason the virulence genes of 6 isolated coagulase positive *Staph. aureus* were examined by PCR and the results showed the presence of enterotoxin producing genes (A, C, D and E) in *Staph. aureus* the three *isolates of Staph. aureus* isolated from luncheon showed the presence of enterotoxin gene 1^{st}(A, E) , 2^{nd} (A, D) and 3^{rd} (B, D). The isolate of *Staph. aureus* isolated from burger showed presence of enterotoxin genes (A and E), the sausage isolate showed the presence of enterotoxin gene (A) while shawerma isolate showed presence of enterotoxin genes(A) as shown in Table (I) (Photo No. 1). The result agree with Eldaly *et al.* (2014) who found that luncheon samples harbored *seb* gene s while burger samples harbored *sed* gene also Tharwat and Elabbasy (2014) reported that SEA enterotoxin gene was the predominant enterotoxin genes which were detected in examined chicken burger and chicken luncheon.

Staph. aureus enterotoxin were analyzed from ready to eat products including pork ham, chicken cold cuts, pork sausage, salami and pork luncheon meat in a study conducted by Fijalkowski *et al*. (2016), this study reported that the most prevalent enterotoxin genes were sei (36%), seln (32%) and encoding exfoliative toxin A (37%). Another study conducted by Puah *et al*. (2016) revealed an incidence of (96.2%) virulence genes from *Staph. aureus* isolated from 200 food samples. A total of 30.8% of the isolates carried *SE* gene which cause food poisoning meanwhile the most common enterotoxin genes found were *seg* (11.5%) and *egc* (5.8%). On the other hands *Inv* A and *stn* virulence genes in the isolated *S. Typhimurium* were positive. *Inv*A gene was amplified and detected at 284 bp while *stn* gene detected and amplified at 617 bp. In Korea, Li *et al*. (2006) could detect 17 virulence genes from isolated *Salmonella* using PCR assays, 14 genes assayed (82.4%) out of these 17 genes included *invA* gene.

The virulence genes of shiga toxin (*stx*1 and *stx*2) were examined using PCR in the four *E. coli* isolates the results were positive for these genes Table (2) (Photo No. 2), these results were nearly similar to Balague *et al*. (2006) who collected 500 food samples from shops selling ready to eat foods in Argentina and *E. coli* virulence gens were examined by multiplex PCR (*stx*1, *stx*2, *eae* A, *cnf*1, *cnf*2, *ein* v, *Lt1*, *ST1* and *ST11*), ten *E. coli* isolates showed the presence of *stx*1, *stx*2 genes while other genes were negative. Another study carried by Bohaychuck *et al.* (2006) reported shiga toxin producing *E. coli* O22: H8 from beef samples in Alberta and Canada.

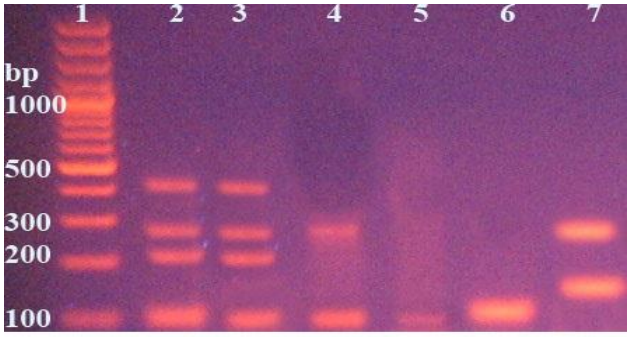

Photo No. (1): Agarose gel electrophoresis of *Staph. aureus* PCR products using enterotoxins Staphylococcus primer.
Lane "1": 100 bp DNA ladder
Lane "2 ": positive amplification of 102 bp for enterotoxin A, 209 bp for enterotoxin E, 278 bp for enterotoxin D and 451 bp for enterotoxin C
Lane "3": positive amplification of 102 bp for enterotoxin A, 209 bp for enterotoxin E, 278 bp for enterotoxin D and 451 bp for enterotoxin C
Lane "4": positive amplification of 102 bp for enterotoxin A and 278 bp for enterotoxin D
Lane "5": positive amplification of 102 bp for enterotoxin A
Lane "6": positive amplification of 102 bp for enterotoxin A
Lane "7": posistive amplification of 164 bp for enterotoxin B and 278 bp for enterotoxin D

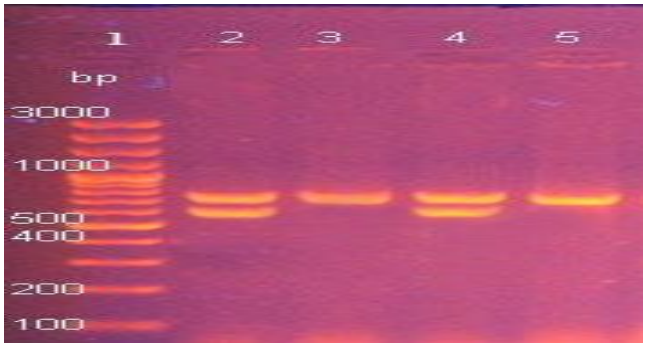

Photo No. (2): Agarose gel electrophoresis of and *E. coli* PCR products using *stx1 and stx2* primers.
Lane "1": 100 bp DNA ladder
Lane "2 ": positive amplification of\614 bp for stx1 gene and 779 bp for stx2.
Lane "3": positive amplification of 779 bp for stx2.
Lane "4": positive amplification of \614 bp for stx1 gene and 779 bp for stx2.
Lane "5": positive amplification of 779 bp for stx2.

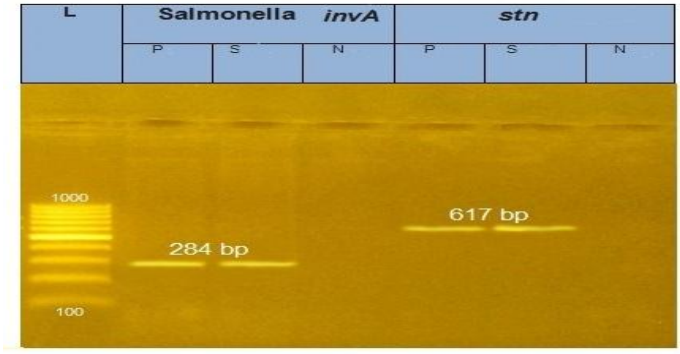

Photo No. (3): Agarose gel electrophoresis of *Salmonella* and PCR products using *inv*A, and *stn*, primers
L= 100 bp DNA ladder**.**
N= negative control.
P= positive control (give amplificationat 617pb for *stn* gene, 284 bp for *inv*A, 614 bp
Sample of S.Typhimurium showed 284 bp amplification for *inv*A gene and 617 pb for *stn* gene.

CONCLUSION

This study confirms that chicken meat products may serve as a source of foodborne pathogens and accordingly a potential public health hazard. Corrective action needs to be employed to minimize the risk of consuming this type of fast food, such action must aim to minimizing the bacterial contamination during the production of chicken meat products (cleaning, cutting, seasoning and stacking), its cooking and serving. Regular surveillance by the public health regulatory bodies will ensure compliance with WHO and ISO standards for food safety.

Also, handling, storage and processing steps are major avenue for the cross contamination of the major materials used for the preparation of such product. Personal hygiene and processing practice of the food vendors are major factors.

REFERENCES

Ahmed, M. (1992): Incidence and Occurrence of *Salmonella* and *E.coli* in packed meat products in Assiut. M.V.Sc. thesis, Fac. of Vet. Med. Assiut University.

AL-Ghamdi, A.Y. (2012): Incidence of Staphylococcus aureus contamination of marketed processed chicken products with special refercence to its antibiotic sensitivity collected from Al Baha city markets, Saudi Arabia. Pak. J. Food Sci. 22;3: 167-169.

Amal, A.S.A.T. (2004): Trials for inhibition of some food poisoning microorganisms in meat products. Ph. D.V. Sci. Fac. Vet. Med. Cairo Univ.

Armany, G.A.; Ibrahim, M. Hemmat; Amin, R.A. and Ahmed, A. Hanaa (2016): Detection of some foodborne pathogens in meat products by Polymerase Chain Reaction. Benha Vet. Med. J. 30, 1; 323-330.

Balague, C.; Khan, A.A.; Fernandez, L.; Redolfi, A.L.; Aquili, V.; Voltattorni, P.; Hofer, C.; Ebner, G.; Duenas, S. and Cerniglia, C.E. (2006): Occurrence of non O157 shiga toxin producing *E.coli* in ready to eat food from supermarkets in Argentina. Food Microbiol; 23(3): 307-313.

Bergadol, M.S. (1989): S. aureus in bacterial food borne pathogens, Editor Doyle M.P. Marcel Dekker press New York Occurrence of food borne pathogenic bacteria in refrigerated chicken meat. Hygiene Alimenter, 16, 100; 97-101.

Bohaychuck, V.M.; Gensler, G.E.; King, R.K.; Manninen, K.I.; Sorensen, O.; Wu, J.; Stiles, M.E. and McMullen, L.M. (2006): occurrence of pathogens in raw and ready to eat meat and poultry products collected from the retail market place in Edmonton, Alberta, Canada. J food Prot; 69(9): 2176-2182.

Cebedo, O.L.; Picart-i-Barrot, L. and Teixdoi-Canelles, A. (2008): Prevalence of Listeria monocytogenes and *Salmonella* in ready – to – eat food in Catalonia. Spain. J. Food Prot., 71; 4: 855-859.

Chiang, Y.C.; Liao, W.W.; Fan, C.M.; Pai, W.Y.; Chiou, C.S. and Tsen, H.Y. (2008): PCR detection of Staphylococcal enterotoxins (SEs) N,O,P,Q, R,U and survey of SE types in S. aureus isolates from food poisoning cases in Taiwan. Int. J. Food.

Chomavarin, C.; Chantarasuk, Y.; Srigulbur, S.; Chareonsudjai, S. and Chaicumper, K. (2006): Enteropathogenic bacteria and enterotoxin-producing *Staph. aureus* isolated from ready to eat food in Khon Kaen, Thailand. Southeast Asian J. Trop. Med. Public Health, 37; 983-990.

Dipineto, L.; Santaniello, A.; Fontanella, M.; Lagos, K.; Fioretti, A. and Menna, L.F. (2006): Presence of Shiga toxin-producing Escherichia coli O157:H7 in living layer hens. Letters in Applied Microbiology 43 (2006) 293–295.

Donelly, C.B.; Leslie, J.E.; Black, L.A. and Lewis, K.H. (1967): Serological identification of enterotoxigenic Staphylococci in cheese. Appl. Microbiol.15; 1382-1389.

EL Jakee, J.; Nagwa, S. Ata; Sherein I. Abd EL-Moez; Mai, M. Kandiel and Nermin, M. Radwan (2014): Assessment of the Prevalence of *Salmonella* in food. Int. J. of Current Microbiology Applied Sciences 3; 3: 30-42.

Eldaly, A. Elsaid.; El Shopary, F. Nermeen and El Bayomi, Rasha (2014): Detection of enterotoxigenic *S. aureus prevalent genes* in some meat products using Multiplex PCR. The 1st International Conference on Impact of Environmental Hazards on Food Safety Faculty of Veterinary Medicine- Zagazig University, 20th August, 2014.

EL-Khatieb, T. (1997): Microbiological status of Egyptian salted meat and fresh sausage. J. of Food Safety, 17 (3); 141-150.

El-Mossalami, H.H.A.; Abd-EL-Rahman, A.A. and Magdy, E.M. (2009): A study on the effect of Garlic and Nigella sativa on some food poisoning bacteria isolated from ready to- eat meat sandwiches in Alexandria City. Assiut Veterinary Medical Journal. 54; 119, 140-158.

Erol, I. and Iseri, O. (2004): Staphylococcal enterotoxins, Ankara Univeritesi Veteriner Fakultesi Dergisi, 51, 3; 239-245.

Fathi, S.M.; Rashwan, M.R. and EL-Said, S.I. (1992): Determination of coliforms and *E.coli* in some meat products using most probable number technique. Assiut Vet. Med. J. 24 (55): 180-185.

Fatin, S.H. (2004): Bacterial hazards associated with consumption of some meat products. Benha Vet. Med. J. 15 (2); 41-54.

Fawzy, A.A. (2004): Sudies on cooked meat and chicken products. Ph.D. Fac. of Vet. Med. Zagazig Univ. Benha branch.

FDA (2002a): Food and Drug Administration. Bacteriological Analytical Manual. 9th Ed., AOAC International, Arlington, VA, USA.

FDA (2002b): Food and Drug Administration: Enumeration of coliform bacteria and *E.coli*. Bacteriological Analytical Manual. Chapter4.

Fijalkowski, K.; Peitler, D. and Karakulsuka, J. (2016): Staphylococci isolated from ready to eat meat identification, antibiotic resistance and toxin gene profile. Int J. food Microbiol.; 238:113-120.

Food and Agriculture Organization (FAO) (2013): Poultry Development Review: Poultry and poultry products: Risks for human health by M Ventura da Silva, FAO Document Repository. Available at: http://www.fao.org/docrep/019/ i3531e/i3531e.pd

Griffin, PM. and Tauxe, RV. (1991): The epidemiology of infections caused by Escherichia coli O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev; 13: 60–98.

*Hamilton, Holds, G.; Hols, G.; Lorimer, M.; Pointon, A. and Sumner, J. (2009):*Micrbiology of beef sausages at retail in Australia. Food Australia.61; 12, 532-533.

Harakeh, S.; Yassinea, H.; Ghariosb, M.; Barbourc, E.; Hajjara, S.; El-Fadeld, M.; Toufeilib, I. and Tannous, R. (2005): Isolation, molecular characterization and antimicrobial resistance patterns of *Salmonella* and Escherichia coli isolated from meat-based fast food in Lebanon. Sci Total Environ 341: 33 - 44.

Ibrahim, M.I.A. (2009): Bacteriological quality and shelflife of some products. M.V. Sc. Thesis. Meat Hygiene, Fac. Vet. Med. Alex. Univ. in fast food with special reference to its enterotoxigenicity. Assiut Vet. Med. J. 54,112; 37-50.

ISO, 16649/2 (2001): Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for the enumeration of beta - glucuronidase - positive *E. coli*: part 2: colony-count technique at 44°C using 5-bromo -4-chloro-3-indoly beta-glucuronide

ISO, 6579, (2002): Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for the Detection of S. spp.

ISO, 6887/2 (2003): Microbiology of Food and Animal Feeding Stuffs-Preparation of Test Samples, Initial Suspension and Decimal Dilutions for Microb. Exam Egypt. J. Chem. Environ. Health, 1 (1):686-69

Kaneko, K.; Hayashidani, H.; Ohtomo, Y.; Kosuge, J.; Kato, M.; Takahashi, K.; Shiraki, Y. and Ogauwa, M. (1999): Bacterialcontamination of ready to eat foods and fresh products in retail shops and food factories. J Food and Drug Analysis 1: 105-115.

Kozacinski, L.; Drosinos, E.; Caklovica, F.; Cocolin, L.; Gasparik-Reichandt, J. and Eskovic, S. (2008): Investigation of microbial association of traditionally fermented sausages. Food Technology and Biotechnology.46; 1,93-106.

Lee, A.; Smith, S.C. and Coloe, P.J. (1998): Survival and growth of Campylobacter jejuni after artificial inoculation onto chicken skin as a function of temperature and packaging conditions, J. Food Protection, 61: 1609-1614.

Li, Q.; Skyberg, J.A.; Fakhr, M.K.; Sherwood, J.S.; Molan, L.K. and logue, C.M. (2006): Antimicrobial susceptibility and characterization of *Salmonella* isolates from processed Bison carcass. APL Microbiol 72: 3046-3049.

Matossian, R. and Kingcott, E.W. (1979): The dona kebab a possible food poisoning hazard. Environm. Health. 86; 67-68.

Mehrotra, M.; Wang, G. and Johnson, M.W. (2000): Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxin, exfoliative toxins, toxic shock syndrome toxin 1, and Methicillin resistance. Journal of Clinical Microbiology, Vol. 38: 1032-1035.

Motten, Vanessa; Fisch Elisa and Cardoso Marisa (2011): Microbial contamination of luncheon meat sliced and packaged in supermarkets in Porto Alegre, Brazil. Acta Scientiae Veterinariae, 39; 1: 940-945.

Mousa, M.M.; Awad, H.A.; Yassien, M.M. and Gouda, H.I. (1993): Microbial quality of some meat products.Vet. Med. J. Giza. 41; 3,59-62.

Murugkar, H.V.; Rahman, H. and Dutta, P.K. (2003): Distribution of virulence genes in *Salmonella* serovars isolated from man and animals. Indian J. Med. Res., 117:66-70.

Oh, S.K.; Lee, N.; Cho, Y.S.; Shin, D.B.; Choi, S.Y. and Koo, M. (2007): Occurrence of toxigenic *Staph. Aureus* in ready to eat food in Korea. J. Food Protection, 70; 1153-1158.

Oliveira, S.D.; Rodenbusch, C.R.; Ce, M.C.; Rocha, S.L.S. and Canal, C.W. (2003): Evaluation of selective and non-selective en-richment PCR procedures for *Salmonella* de-tection. Lett. Appl. Microbiol., 36: 217-221.

Osek, J. and Wieczorek, K. (2010): Zoonosis and their etiological agents in the EFSA report for 2008. ZycieWeterynaryjne. 2010. 85: 4, 315-324.

Pelczar, MJ.; Chane, CS. and Kreig, NR. (2006): Microbiology 5th edition. Tata McGraw-Hill Publishing Company Limited, New Delhi.

Puah, S.M.; Chua, K.H. and tan, J.A. (2016): virulence factor and antibiotic susceptibility of

Staph. Aureus isolates in ready to eat foods: detection of *Staph.* aureus contamination and a high prevalence of virulence genes. Int. J. Environ Res public health 5; 13(2)199.

Saleh, E.A.; Ali, H.A. and Abu-Khadra, A.M. (2010): Detection of some food poisoning microorganisms in some meat products. Alex. J. Vet. Sc. 31; 1, 27-33.

Samaha, I.A.; Ibrahim, H.A.A. and Hamada, M.O. (2012): Isolation of some enteropathogens from retailed poultry meat in Alexandria Province. Alex. J. Vet. Science 37; 1: 17-22.

SAS Institute (2003): SAS Proprietary Software. Re. 9.1. SAS Instit. Inc. Cary, NC.

Shalaby, M. Amany and Zaki, M.S. Eman (2008): Occurrence of Staphylococcus aureus in fast food with special reference to its enterotoxigenicity. Assiut Vet. Med. J. 54,112: 37-50.

Shawish, R.R. and AL-Humam, N.A. (2016): Contamination of beef products with Staphylococcal classical enterotoxin in Egypt and Saudi Arabia. Hygiene and Infection Control, 11; ISSN 2196-5226.

Soultos, N.; Abrahim, A. and Abrosiadis, I. (2003): Incidence of some food borne bacterial pathogens in northern Greece. Archiv *Staph.* Furer Lebensmittel. Hygiene 54,(3);55-57.

Tharwat, A. Elsayed and Elabbasy, M. Tharwat (2014): Classical Staphylococcus aureus Enterotoxins genes in some chicken meat products marketed in Zagazig city, Egypt. The 1st International Conference on Impact of Environmental Hazards on Food Safety Faculty of Veterinary Medicine- Zagazig University, 20th August, 2014.

Tsang, O. (2002): Guidelines for Ready-To-Eat Food. Road and Environmental Hygiene Department, Hong Kong.pp.15 – 16

Tudor, L.; Togoe, I. and Mitranescu, E. (2010): The microbiological quality analysis of some meat products traded on Bucharest markets. Lucrari Stiintifice-Universitatea de Stiinte Agricole a Banatului Timisoara, Medicina Veterinara. 40; 688-693.

World Health Organization (WHO) (2013): MEDIA Centre, Fact sheet N°139: *Salmonella* (non-typhoidal), Available at: http://www.who. nt/ media centre/ factsheets/fs139/en.

مدى تواجد جينات الضراوة ببعض البكتيريا المنقولة بالغذاء في بعض منتجات لحوم الدواجن

نشوى محمد ذكى ، حاتم فتحى احمد الدسوقى ، وفاء محمد عبد الخبير جاد

Email: nashwazaki80@gmail.com Assiut University web-site: www.aun.edu.eg

تم جمع ٢٠٠ عينه من لانشون وبيرجر وسجق وشاورما الدجاج بواقع ٥٠ عينه لكل منها. حيث تم عمل فحص بكتيري لكل من الاستاف اوريس والايشيرشيا كولاى وكذا معرفة مدى تواجد ميكروب السالمونيلا حيث كانت نسب العزل لميكروب الاستاف اوريس كالتالي ٦% و ٢% و٢ % و٢% بينما نسب العزل للالايشيرشيا كولاى كالتالى ٢% و ٤% و٠% و ٢% على الترتيب وتم عزل عترة السالمونيلا تيفيموريم من شاورما الدجاج بنسبة ٢ %. حيث تبين من هذه الدراسة ان عينات الانشون والسجق والهامبورجر كانت خالية من السالمونيلا عند الفحص البكتيريولوجي . تم عزل٦ معزولات من ميكروب الاستاف اوريس (الموجبة لتجلط البلازما).
تنتج المكورات العنقودية الذهبية مجموعة واسعة من السموم المعوية والتي تقوم بإثارة مراكز طالقئ في المخ وتشكل أحد الأسباب الرئيسية للتسمم الغذائي، والذي يحدث عادة بعد تناول الأطعمة المختلفة، لا سيما منتجات لحوم الدجاج الملوثة بالمكورات العنقودية الذهبية عن طريق سوء التعامل والتخزين في درجات حرارة مرتفعة بالإضافة لما تسببه كل من ميكروب السالمونيلا والايشيريشياكولاى من حالات الإسهال الحاد لذلك تم تي تؤثر على قدرة الميكروب على احداث حالات مرضية عند تناول الاطعمة الملوثة بهذه الميكروبات. عند فحص السالمونيلافحص جينات الضرواه لكل منهما. واجراء اختبار تفاعل البلمرة المتسلسل لتحديد وجود جينات الضراوة في الميكروبات المعزولة وال تيفيموريم المعزولة اظهرت وجود جيني ,invAstn. وبفحص معزولات ميكروب الايشيرشياكولاى المعزولة تواجد بها جيني stx2 , stx1 كما أثبتت تواجد معظم جينات الضراوه لميكروب الستاف اوريس المعزول من العينات. وقد نقشت الأهمية الصحية للمعزولات وكذلك كيفية الإقلال من تواجدها باتباع نظم إدارة سلامة الغذاء أثناء التصنيع.