



Detection of virulent genes of Staph aureus and E.coli in fresh, frozen chicken and Ready-to- Eat chicken meat products

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Abstract

Enterotoxigenic Staph. aureus is one of the causative agents of food borne intoxication. For this reason, determination of its prevalence in foods is important with respect to assessing public health risk. Many different types of foods are considered as a source of the bacterium and have been identified as a potential source of shiga Toxin-producing Escherichia coli. The research study focused on detection of the virulent genes of Staph. aureus enterotoxins and shiga toxins of E.coli in fresh and frozen chicken and ready- to- eat chicken meat products (RTE), by using Multiplex PCR (m- PCR) technique. It was carried out on 20 isolates of Staph. aureus from different ready- to- eat chicken meat products (14 isolates) as well as fresh and frozen chicken carcasses (six isolates; three of each), the (Sea) gene of Staph. aureus had the highest prevalence of virulence genes. Shiga toxin 1 (Stx1) gene could be detected only in one strain (50%) isolated from both of chicken shawarma and chicken salad. Shiga toxin 2 (Stx2) could be detected in two isolates of E. coli from chicken Fajitas and chicken salad. The public health significance of both toxins was discussed.

(Key words: Ready to eat, enterotoxins producing genes, m-PCR, staph. aureus, shiga toxin).

Introduction

Staphylococcal enterotoxins (SEs) produced by Staph. aureus are the most recognizable bacterial super antigenic toxins causing food poisoning in humans throughout the world. It remains unclear how SEs induce emesis and its emetic signal pathway (Mori, et al. (2007). Staphylococcal food poisoning outbreaks which are very common across the world, more than 100 children and adults suffered from the typical symptoms of (SFP) and required hospitalization. Food and clinical samples were found to contain a large number of enterotoxigenic Staph. aureus. All enterotoxigenic isolates produced a combination of (Seb) and (Sed) enterotoxins and were sensitive to oxacillin and vancomycin (Nema, et al. (2007). Staph. aureus is one of the major pathogens that can cause Staphylococcal intoxication and food poisoning (Lin, et al. (2009). There are five major classical types of Staphylococcal enterotoxins (SEs): SEA, SEB, SEC, SED, and SEE, encoded by sea, seb, sec, sed and see, respectively (Pinchuk et al., 2010). The concentration of Staph. aureus necessary to cause food poisoning ranges from 10^6 to 10^8 cfu/g in food samples, and for sensitive persons even 10^5 cfu/g of staphylococcal bacteria are capable of producing enough SEs (around 1 μ g) to generate symptoms (Alarcon et al., 2006). There are many factors affecting enterotoxin production in food such as cell count, salt concentration, pH, temperature and presence of competitive flora (Pelisser et al., 2009). Staphylococcus enterotoxins could be found in the ready -to -eat

meat after 43 hours at 22°C. Several reports have described the development of multiplex polymerase chain reaction technique (multiplex PCR) protocols for the detection of SEs genes (Mehrotra et al., 2000 and Omoe et al., 2002). For the newer SEs, specific PCR for detection of genes in food has been developed (Omoe et al., 2005 and Chiang et al., 2008). The strains of Shiga toxin-producing E. coli (STEC) caused a large outbreak in Europe in 2011, such raw or under cooked foodstuffs get contaminated either during primary production as slaughtering or further processing and handling (e.g. cross contamination during processing, human-to-food contamination via food handlers), E. coli has been isolated worldwide from poultry meat (Adesiji et al., 2011). Many different types of foods are sources of E. coli and have been identified as a potential source of shiga Toxin-producing Escherichia coli (STEC), (CDC, 2015). There are two major Shiga toxin gene families stx1 and stx2 and multiple Shiga toxin genotypes within each family. The stx genotype is unrelated to the in vitro cytotoxicity or quantity of Shiga toxin produced, but does appear to be associated with the severity of clinical illness (Orth et al., 2007). Stx2 is statistically associated with severe symptoms, including bloody diarrhea (Zhang et al., 2007 and Persson et al., 2007). Therefore the present study planned to detect the virulent genes of Staph. aureus and E. coli in fresh, frozen chicken and ready to eat chicken meat products.

Material and Method:

A total of 120 collected samples of RTE chicken meat products, fresh and frozen chicken were

collected from different restaurants in Cairo and Giza Governorates and aseptically transferred in

its original containers without delay to the laboratory and subjected to sample preparation according to **ISO 6887-2 (2003)** for further examinations:

1- Isolation of *Staphylococcus aureus* according to **FDA, (2001)** by Baird Parker media at 35°C for 24-48hrs.

2- Isolation of *E. coli* according to **FDA, (2002)** by Eosin Methylene Blue (EMB) at 35°C for 18 hrs.

3- Detection of *Staphylococcus aureus* enterotoxins genes by m PCR.

4- Detection of *E. coli* shiga toxins by m PCR.

Detection of *Staphylococcus aureus* enterotoxins genes by m PCR:

Oligonucleotide primers used for mPCR according to **Naresh et al., (2000)**, sets of primers were used for of *Staph. aureus* enterotoxins (A, B,C, D and E) by multiplex polymerase chain reaction as described in Table (a).

Master Mix	25 µl/ reaction
D.D. H ₂ O (autoclaved)	1.5 µl
2x mix of mPCR kit (Qiagen)	12.5 µl
Primer 1 forward (20pm/ µl)	3 µl
Primer A Reverse (20pm/ µl)	0.5 µl
Primer B Reverse (20pm/ µl)	0.5 µl
Primer C1 Reverse (20pm/ µl)	0.5 µl
Primer C2 Reverse (20pm/ µl)	0.5 µl
Primer D Reverse (20pm/ µl)	0.5 µl
Primer E Reverse (20pm/ µl)	0.5 µl
Template DNA	5µl

Gently vortex the samples and spin down. PCR tubes were transferred and then placed in thermo cycler (BioRad). The following thermal profile ***Staph. aureus*** enterotoxins (A, B, C, D, E):

Initial denaturation at 95°C for 3 minutes:94°Cfor 30 second, 50°C for 30 second, 72°C for 30 Second for 25 cycles for each. The final extension at 72°C for 10 minutes, then hold at 4°C.

Detection of *E. coli* shiga toxins by m PCR:

DNA extraction:

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) . Briefly, 200 µl of the sample suspension was added to 20 µl of proteinase K and 200 µl of lysis buffer and incubated at 56 o C for 10 min in a Biometra Tsc thermal block. After incubation, 200 µl of 100 % ethanol was added to the lysate and votexed. The sample was then washed twice and centrifuged according to the

DNA extraction:according to (Franco et.al, 2008):

Loopful of isolated *Staph. aureus* was suspended in 50 µl of distilled water. The cellular suspension was brought to boil during 10 minute and immediately was centrifuged at 14,000 RPM for 5 min. the supernatant was directly used for PCR assay.

PCR amplification:

For each primer take 2 µl of the stock (primer 1) with 8 µl autoclaved D.D. H₂O and also for (primer 2) to be 20 pmol/µl. Gently vortex and briefly centrifuge 2x multiplex PCR kit Qiagen after thawing.A thin-walled PCR tube was Place on ice and the following components was added for each 25-µl reaction.

manufacturer's instructions. DNA was eluted with 100 µl of elution buffer supplied in the kit.

PCR amplification:

A 25- µl master mix reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of 20 pmol conc. of each primer, 4.5 µl of water, and 6 µl of template DNA. The reactions were performed in applied bio-system 2720 thermal cyclers.

Analysis of the PCR Products:

Fifteen microliters of each PCR product were loaded in each gel lane of 1.5% agarose gel (Applichem). Electrophoresis was done in 1x TBE buffer using 5V/cm gradients. A Gelpilot 100 bp plus DNA Ladder (Qiagen) was used to determine the fragment sizes. The PCR photos were photographed and analyzed using a gel documentation system (Alpha Innotech, Biometra, Germany) through its computer software.

Results

Table (a):Nucleotidesequencesandanticipatedsizesof PCRproducts for the Staph. aureusenterotoxins genes-specificoligonucleotideprimers.

Primername and size	Description	Nucleotide sequence	PCR product size (bp)	Reference
SA-A	Reverse primer for sea	ATTAACCGAAGGTTCTGT	270	Betley and Mekalanos (1988)
SA-B	Reverse primer for seb	ATAGTGACGAGTTAGGTA	165	Jones and Khan (1986)
SA-C	Reverse primer for sec	AAGTACATTTTGTAAGTTCC	69	Bohach and Schlievert (1987)
SA-D	Reverse primer for sed	TTCGGGAAAATCACCTTAA	306	Bayles and Iandolo (1989)
SA-E	Reverse primer for see	GCCAAAGCTGTCTGAG	213	Couch, et al. (1988)

Table (1): Prevalence of Staph. aureus genes isolated from examined RTE chicken meat products .

Type of chicken meat product	No. of tested strains	Enterotoxigenic genes of Staph. aureus							
		Single producers						Multiple producers	
		Sea		Seb		Sec		Seb & Sec	
No.	%	No.	%	No.	%	No.	%		
Shawarma	--	--	0.0	--	0.0	--	0.0	--	0.0
Grilled chicken	2	1	50	--	0.0	--	0.0	--	0.0
Chicken Pane	2	--	0.0	1	50	--	0.0	--	0.0
Fajita	2	--	0.0	--	0.0	--	0.0	1	50
Shish tawoak	2	1	50	--	0.0	--	0.0	1	50
Chicken Burger	2	1	50	--	0.0	--	0.0	--	0.0
Chicken Salad	2	1	50	--	0.0	--	0.0	1	50
Chicken Mandy	2	--	0.0	--	0.0	1	50	--	0.0
Fried chicken	--	--	0.0	--	0.0	--	0.0	--	0.0
Pop chicken	--	--	0.0	--	0.0	--	0.0	--	0.0
Fresh chicken	3	--	0.0	--	0.0	--	0.0	1	33.33
Frozen chicken	3	--	0.0	--	0.0	--	0.0	--	0.0

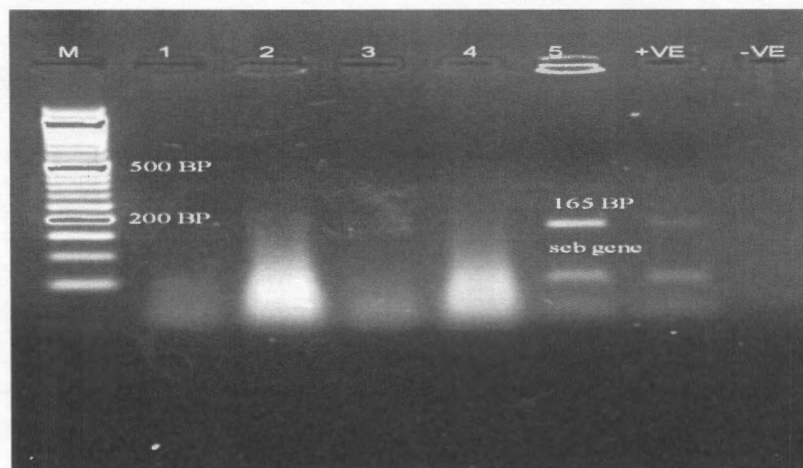


Fig. (1): SebStaph. aureus enterotoxin gene isolated from chicken pane.Lane M: 100 bp ladder as molecular size DNA marker.Lane (5) positive amplification of 165 bp for Seb gene .+ VE = Positive control.- VE = Negative control.

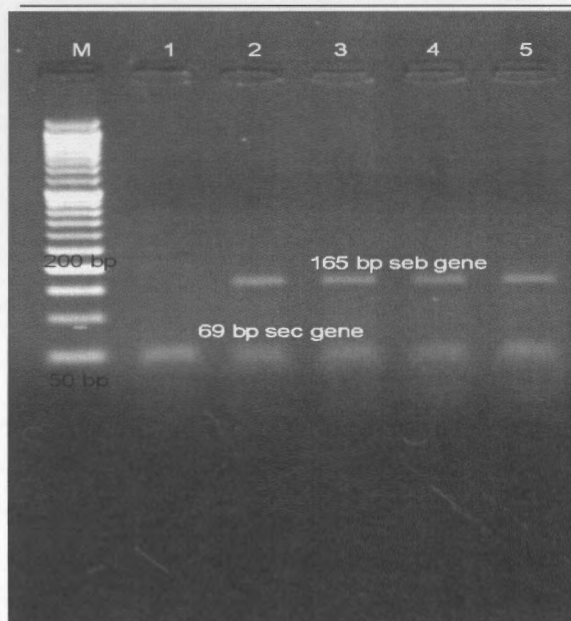


Fig. (2): Seb&SecStaph. aureus enterotoxin genes isolated from different RTE chicken meat products.Lane M: 100 bp ladder as molecular size DNA marker.Lanes (2, 3, 4 & 5) positive amplification of 165 bp for Sseb gene.Lanes (1, 2, 3, 4 & 5) positive amplification of 69 bp for Sec gene.

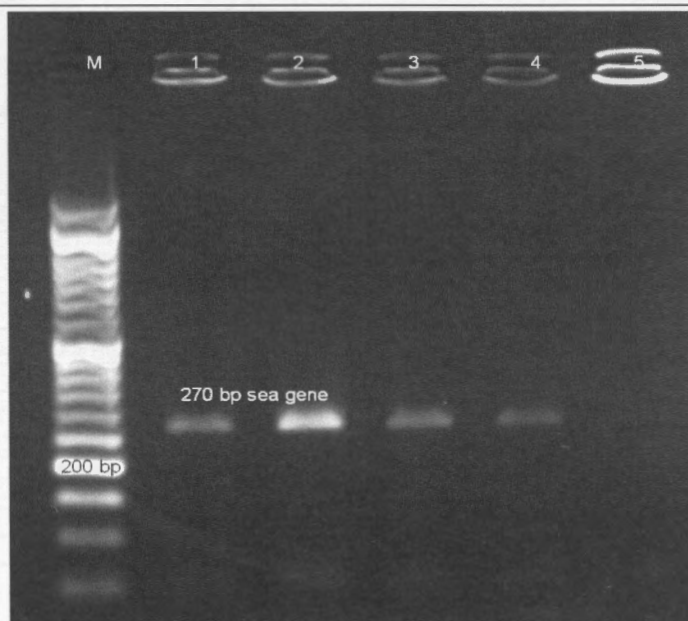


Fig. (3): Staph. aureus enterotoxin gene A (Sea) isolated from different RTE chicken meals.Lane M: 100 bp ladder as molecular size DNA marker.Lanes (1, 2, 3 & 4) Positive amplification of 270 bp for Sea gene.Lane (5) Negative control for enterotoxin A.

Table (2) Prevalence of isolated E. coli and Shiga toxin genes (Stx) in examined fresh, frozen and RTE chicken meals using multiplex PCR.

Type of chicken meat product	No. of isolated strains	Virulent genes of E. coli			
		Shiga toxin 1 (Stx1)		Shiga toxin 11 (Stx2)	
		No.	%	No.	%
Shawerma	2	1	50	---	0.0
Grilled chicken	1	---	0.0	---	0.0
Chicken pane	1	--	0.0	--	0.0
Fajitas	3	1	33.33	1	33.33
Shish tawoak	1	--	0.0	--	0.0
Chicken burger	--	--	0.0	--	0.0
Chicken Salad	2	1	50	1	50
Chicken mandy	--	--	0.0	--	0.0
Fried chicken	--	--	0.0	--	0.0
Pop chicken	---	---	0.0	---	0.0
Fresh chicken	3	1	33.3	---	0.0
Frozen chicken	3	1	33.3	---	0.0



Fig. (5): Stx1 gene of *E. coli* strains isolated from examined chicken meat products. Lanes (1, 2, 3,4&5): Positive amplification of 180 bp for Stx1 gene. Lane (6) : Negative control for Stx1 gene. C- : Positive control for Stx1 gene.

Discussion

Results in **Table (1)** illustrated the incidence of isolated organisms *Staph. aureus*. and *E. coli*, which could be detected in 3 (30% each) of examined fresh and frozen chicken samples. In this regard, **Islam (2014)** could isolate coagulase positive *Staph. aureus* from 16.67% of the frozen chicken samples. Many investigators discussed the rate of isolation of *E. coli* from fresh chicken, 19% prevalence was observed in South Africa (**Dahal, 2007**), 48.4% in Morocco (**Cohen et al., 2007**) and as high as 98% in India (**Saikia and Joshi, 2010**). In Nigeria, 16% (**Adesiji et al., 2011**) and 11.1% in Calabar metropolis (**Ukut et al., 2010**). Considering the incidence of *E. coli*, in frozen chicken carcasses, lower findings represented by 6(12%) and 5(10%) of imported frozen chicken and locally frozen chicken respectively, was reported by **Elnawawi, et al. (2012)**. **Moharum (2005)** and **Mansour and Basha (2009)** could isolate *E. coli* from 8% and 6% of locally frozen chicken and imported frozen chicken, respectively. Higher prevalence of *E. coli* was reported by **El-Nasri et al., 2005 (65%)**, **Adeyanju and Ishola, (2014) (47.2%)**, out of 53 examined frozen chicken samples. **Table (1)** also showed that *Staph. aureus* could be isolated from grilled chicken, chicken pane, shish tawoak, fajita, chicken burger, chicken meat salad and chicken mandy with two strains (20%) of each. While *E. coli* could be isolated from two sample of each (20%) of chicken shawerma and chicken salad, one sample (10%) of each grilled chicken, chicken pane and shish tawoak, as well as from three samples (30%) from chicken fajita and chicken meals, respectively. Similar results for *staph. aureus* (20%) was reported by **Eid, et al.,**



Fig. (6): Stx2 gene of *E. coli* strains isolated from examined chicken meals. Lanes (1 & 2): Positive amplification of 255 bp for Stx2 gene. -VE: Negative control for Stx2 gene.

(2014), **Abo El-Enaen, et al. (2012) (24%)** and **Karmi (2013) (20%)**, while lower result was mentioned by **Farag (2009) (5.9%)**, and **Cohen, et al., (2007) (10.4%)**. Higher values were recorded by **Kelman, et al. (2011) (29%)** and **Eid, et al., (2014) (52%)**. Regarding prevalence of *E. coli*, **Eid, et al. (2014)** failed to detect *E. coli* from examined shish tawoak samples, which agreed with this study, moreover, **Abo El-Enaen, et al. (2012)** and **Ahmed and Shimamoto (2014)** mentioned that *E. coli* outbreak in USA, traced to Costco chicken meat salads appears to have been caused by vegetables in the salad, rather than the chicken itself, according to company officials. As well as, *E. coli* may be used as an indicator microorganism because it provides an estimate of fecal contamination and poor sanitation during processing (**Eisel et al., 1997**). The presence of *E. coli* in high numbers indicates the presence of organisms originating from fecal pollution. This is due to improper slaughtering techniques, contaminated surfaces and/or handling of the meat by infected food handlers (**Nel et al., 2004**). In addition, the presence of these pathogens can be due to contamination taking place during the meat processing at slaughter house or due to the poor handling of the retailers of meat (**Kagambèga et al., 2011**). The results obtained in **Table (a)** illustrated the sets of oligonucleotide primers used for amplification of DNA of *Staph. aureus* virulent genes responsible for enterotoxins types (A, B, C, D and E) production by multiplex Polymerase Chain Reaction (m-PCR). Multiplex PCR technique was carried out on 20 isolates of *Staph. aureus* from different RTE chicken meat products (14 isolates) as well as fresh and frozen

examined grilled, shish tawoak, burger and chicken salad meals (50% of each) corresponding to the total isolates of each product single producer genes, while the gene responsible for production of enterotoxin type B (Seb) was detected in chicken pane only (50%). Moreover, Sec gene was detected in chicken mandy (50%). Multiple genes (Seb & Sec) were detected in three meals including chicken fajitas, shish tawoak and chicken salad (50%). In addition to one isolate contained genes responsible for production of Seb & Sec (33.33%) was detected in examined fresh chicken samples as shown in **Table (2) and Fig. (2, 3, and 4)**. From the obtained results, it could be concluded that *Staph. aureus* failed to be isolated from chicken shawerma, fried chicken, and pop chicken as RTE chicken meat products. Moreover, the isolated organism from frozen chicken was not contained virulent genes responsible for enterotoxin production. Also tested *Staph. aureus* strains in the present study possess no genes responsible for enterotoxins D&E production. The obtained results agreed with **Awadallah, et al. (2014)** who could detect two strains (50%) of enterotoxigenic *Staph. aureus* isolated from RTE chicken luncheon. The isolated strains could able to produce enterotoxins (Sea) and (Sec). In spite of the low prevalence of *Staph. aureus* in RTE meat products, the proportion of enterotoxigenic strains is considered high. In this respect, **Bania, et al. (2006)** reported that 15-80% of *Staph. aureus* strains isolated from various food sources were enterotoxigenic. **Stastkova, et al. (2011)** found that 10 (22.2%) out of 45 examined *Staph. aureus* strains were contained gene responsible for enterotoxin type A production (Sea), Seb (n=8), Sec (n=10), Sed (n=7) and mixed of Seb & Sed (n=1). Moreover, **Sung, et al. (2008)** could detect Sea gene in 25% and 14% from *Staph. aureus* recovered from human and animal isolates, respectively. In this circumstances, **Madahi, et al. (2014)** found that 33.33% out of 72 *Staph. aureus* isolated from chicken nuggets were able to produce Sea, 4.16% Seb, 12.50% Sec, 8.33% Sed, 12.50% Sea & Sec, and 12.50% Sea & Sed. The most commonly detected genes were Sea (25%), Sea (8.33%), Sec (12.50%), both Sea & Sed (12.50%), as well as both Sea & Sec (12.50%). Moreover, in a Turkish investigation conducted by **Gencay, et al. (2010)** showed that only 2.9% of 70 tested *Staph. aureus* strains were positive for Sea gene while there were no positive results for other putative genes. **Akineden, et al. (2001)** showed that 67.96% of *Staph. aureus* isolates had one or more SEs genes

including Sea, Sec, Sed, which was significantly more prevalent than in the present study. **Aydin et al, (2011)** could detect Seb gene in 5 *Staph. aureus* strains (5.4%) isolated from meat and dairy products. Actually, (Seb) was the most important enterotoxin that causes gastroenteritis (**Fooladi et al., 2010**). **Lim, et al. (2004)** determined that 22.28% of *Staph. aureus* isolates harbored Sea, Seb, and Sec genes while Sea was the most frequent SEs gene (86.48%), which was in agreement with our study. **Gilbert and Humphery (1998)** concluded that the source of *Staph. aureus* intoxication is any kind of food which has comes into contact with food handlers' hands contaminated with *Staph. aureus* and subsequently not properly stored. Isolation of enterotoxigenic *Staph. aureus* from nasal and hand swabs of RTE chicken luncheon handlers, substantiate the role played by them in dissemination of such bacteria through foods they handle (**Tremaine et al., 1993**). Moreover, our results are in agreement with those of **Gücükoğlu, et al. (2013)**. **Baumgartner, et al. (2014)** found that Sea gene was most frequent, with a prevalence rate of 16% among all tested strains, followed by Sec (11% of strains), Sed (7%), Seb (4%), and See (1%). In a study conducted by **Balaban and Rasooly (2000)** have shown that one of the most common types of food intoxication is caused by certain Staphylococcal strains, mainly *Staph. aureus* of the many extracellular toxins, Staphylococcal enterotoxins (SE's) pose the greatest risk to consumer's health. These enterotoxins are highly resistant to heat, therefore, measures to prevent the growth of *Staph. aureus* are critical because normal cooking's temperature will not destroy the toxins. Various typing methods have been developed to characterize *Staph. aureus* isolates. **Bryan (1988)** observed that out of 175 staphylococcal outbreaks, 29% were traced to ham and 8% to chicken dishes. Moreover, *Staph. aureus* was detected in beef and chicken luncheon (10% each). In previous study, coagulase positive *Staph. aureus* was detected in 7/110 (6.4%) hamburger patties in turkey (**Ayciek et al., 2004**). Moreover, (**Murray, 2005**) concluded that very small dose of SEs can cause *Staph. aureus* food poisoning. The onset of symptoms (diarrhea and/or food poisoning) is approximately 1-6 hours after ingestion of *Staph. aureus* contaminated food, depending on the amounts of toxin consumed and sensitivity of the individuals to the toxins (**Argudin et al., 2010; Pinchuk et al., 2010; Patel and Myers, 2013**). Gently heated and

uncooked foods may promote *Staph. aureus* contamination from unhygienic hand contact and/or raw materials (Jay, 2000). A previous study conducted in Bangkok reported that Sec or Sea alone, or a combination of them, are frequently found in *Staph. aureus* isolated from food samples (Pumtong et al., 2008). 62 (16.1%) *Staphylococcus* isolates from 385 food samples were tested by polymerase chain reaction (PCR) for five super antigenic toxin genes including four classical enterotoxins Sea, Seb, Sec, Sed, and See. The PCR positive isolates were tested for toxin expression (Hennekinne et al., 2012). *Staph. aureus* was isolated from 4 (13.5%) examined chicken samples were positive for Sec gene (Aye et al., 2014). El-Jakee, et al. (2013) could isolate *Staph. aureus* with incidence of 3 (6%) from examined chicken products, one of them was found to contain the genes responsible for production of both enterotoxin types B&D (Seb&Sed). The author concluded that generally, five classical *Staphylococci* enterotoxin (SE) (Sea to See) are recognized. Regarding *Staph. aureus* genes responsible for toxins production, Demir et al. (2011) examined 120 *Staph. aureus* strains to investigate the presence of genes encoding staphylococcal enterotoxins (SEs), by PCR. The authors could detect Sea from five isolates (4.17%) and Seb from one isolate (0.83%). In this respect, Wongboot, et al. (2015) found that 60% of examined 57 *Staph. aureus* strains isolated from ready-to-eat food (RTE) was positive for presence of super-antigenic toxin genes, out of which 46% were positive for Sea gene. (Sea) was recovered from 77.8% of all SFD outbreaks in the United States followed by Sed (37.5%) and (Seb) (10%) (Balaban and Rasooly, 2000 and Argudin et al., 2010). Sea is the most commonly found enterotoxin among *Staphylococcal* food Intoxication (SFI) outbreaks in Japan, France, and UK (Argudin et al., 2010). However, (Sec) and (See) are also implicated with SFI. In this regard, Syne et al. (2013) added that the counts of *Staph. aureus* increased after heat treatment, and only post cooking and environmental surfaces that came into contact with RTE foods that were

Conclusion

RTE chicken meat products and raw chicken are implicated in *Staphylococcal* food Intoxication (SFI), so all retailers and restaurants should establish, design, improve, imply and update a system for preventive control to improve the safety of their products, known as HACCP (Hazard Analysis and Critical Control Points). Control of Shiga toxin-producing *E. coli* (STEC)

contaminated with *Staph. aureus* during slicing and packaging harbored the organism which is also frequently found on food handler's gloves. Staphylococcal enterotoxins are highly stable and highly heat-resistant and resistant to environmental conditions such as freezing and drying (Le Loir, 2003 and Hennekinne, 2012). They are also resistant to proteolytic enzymes such as pepsin or trypsin and low pH, enabling them to be fully functional in the gastrointestinal tract after ingestion (Le Loir, 2003 and Argudin, 2010). An estimated 0.1 µg of SEs can cause *Staphylococcal* food poisoning in humans (Le Loir, 2003). Table (2) and Fig. (4, 5) showed that not all isolated *E. coli* possess virulent genes. Shiga toxin 1 (Stx1) gene could be detected only in one strain (50%) isolated from both of shawerma and chicken salad meals, such gene detected with 33.3% in each of fajita, fresh and frozen chicken samples. On the other hand, Shiga toxin 2 (Stx2) could be detected in two isolates of *E. coli* from chicken Fajita and chicken salad with incidence of 33.3% and 50%, respectively. Moreover, isolated *E. coli* from grilled, pane and shish tawoak did not contain either Stx1 and/or Stx2 or any other virulent genes. Moreover, *E. coli* isolates from grilled and pane chicken meals did not possess any virulent genes. In this respect, Khatib, et al., (2015) found that 6 isolates (15.39%) of *E. coli* from samples of Lebanese fresh products were positive for gene responsible for production of Stx1 and 2 isolates (5.13) for Stx2. These results were inconsistent with that obtained in the present study. Moreover, Dutta, et al. (2011) examined 42 *E. coli* isolates for the presence of shiga toxin genes (STEC) using multiplex PCR. Multiplex PCR assay yielded amplified products of 180 bp and 255bp for Stx1 and Stx2 respectively. They could detect four strains (9.5%) contained both Stx1 & Stx2, three isolates contained Stx1 only (7.1%) and two isolates found to contain Stx2 (4.8%). In these circumstances, Awadallah, et al. (2014) could detect both Stx1 & Stx2 in one *E. coli* strain (2.5%) isolated from RTE chicken luncheon.

illness in humans requires good slaughterhouse practice and kitchen hygiene and heat treatment of raw meat and milk. STEC is destroyed by heat; (at core temperature of 70°C for 2 minutes) will protect consumers from infection from these sources. It is essential to provide hygienic food handling and good chilled storage conditions to ensure that other foods do not become contaminated. Infected food handlers should be

excluded from working until microbiological clearance of a stool sample has been obtained. Multiplex PCR is considered more sensitive than

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الملخص العربي

تغريد حمدي عباس* خالد شوقي طلحة* ، عادل محمد إبراهيم** و محمد خالد المسلمي**
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يعتبر ميكروب استافيلوكوكاس أورياس المنتج للسم واحدا من أسباب التسمم الغذائي و لهذا السبب من المهم قياس مدى وجوده في الطعام مع الأخذ بالاعتبار تقدير مخاطره على الصحة العامة . تعتبر أنواع كثيرة من الطعام مصدرا للبكتيريا و قد عرفت كمصدر محتمل للإشرشياكولاي المنتجة لسم الشيجا . و قد ركزت الدراسة الحالية على فحص الجينات الضارة لميكروبات الاستافيلوكوكاس أورياس و الإشرشياكولاي في الدجاج الطازج ، و المجمد ، و منتجات الدجاج الجاهزة للأكل باستخدام تفاعل البلمرة المتسلسل . و قد تم إجراء الفحص على عشرين معزولة من ميكروب الاستافيلوكوكاس أورياس من منتجات الدجاج الجاهزة (أربعة عشرة معزولة) الى جانب الدجاج المبرد و المجمد (ستة معزولة و ثلاثة من كل) . و قد وجد ان الجين الاستافيلوكوكاس أورياس لديه اعلى نسبة من الجينات الضارة ، و سم الشيجا 1 و وجد في معزولة واحدة بنسبة (50%) من الشاورما و سلطة الدجاج . و سم الشيجا 2 و وجد في معزولتين من الفاهيتا و السلطة .