

## Incidence of toxigenic genes of *Staphylococcus aureus* isolated from chicken meat

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### Abstract:

Poultry meat is highly palatable, digestible, desirable and nutritious for all ages in addition to low price in comparison to beef meat. Therefore, a total of 100 Fresh and frozen thigh and breast chicken meat samples were collected from Aswan city supermarkets, abattoirs, butcher's shops for evaluation of the incidence of contamination with *Staph. aureus*. The results revealed that the incidence of isolated *Staph. aureus* from frozen chicken thigh, breast, fresh chicken thigh and breast were 40, 36, 32 and 32%; This result at frozen chicken meat more than fresh chicken meat may be due to excess handling of frozen meat and leave to more time at surfaces with un clean utensils before freezing. While *Staphylococcal* count in the same examined samples were  $7.55 \times 10^2$ ,  $5.8 \times 10^2$ ,  $4.11 \times 10^3$ ,  $2.53 \times 10^2$ ; respectively. The results were reversed and therefore modified by confirmation of the results by using Polymerase Chain Reaction (PCR), revealed that fresh chicken thigh samples were positive for *sea* gene of *Staph-aureus* strains, frozen chicken meat samples were positive for *sec* gene of *Staph-aureus* and fresh chicken thigh, frozen chicken breast samples were negative for *sea*, *seb*, *sed* and *sea* genes of *Staph. aureus*.

**Keywords:** Frozen chicken meat, *Staphylococcus aureus*, Staphylococcal count, *sea* genes

### 1- INTRODUCTION

Poultry meat is one of the most important source of animal protein for the majority of Egyptians reach to more than 45 % of total consumption of animal protein (Hosny, 2006). Chicken carcasses have higher pathogenic counts and spoilage than most other foods, where carcass can be contaminated at several points during the processing operation; de-feathering, scalding and evisceration as well as cross contamination from processing equipment and birds (Gonzalez-Fandos and Dominguez, 2006).

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*Staphylococci* are a group of gram-positive, facultative aerobic and always unencapsulated organisms, which are commissioned for infection of different tissues and several diseases. These bacteria are carried mostly transiently by about 20 and 30% of healthy adults on the skin and nares, respectively (Xu et al., 2008). More than 30 different types of *Staphylococci* cause infection for people and its related illness can range from mild to severe form, no treatment required to even potentially fatal. Mainly these infections caused by *Staph. aureus*, which has been regarded as leading issues both in medicine and food safety and can typically make a wide variety of infections, including skin infections and sometimes pneumonia, endocarditis, osteomyelitis, gastroenteritis, toxic shock syndrome and scalded skin syndrome (FDA, 2012).

Poultry meat contamination with food borne pathogens remains an important public health issue as most of food poisoning bacteria contaminate chicken meat (Mbata, 2005). Therefore, the present study aimed to evaluate the bacteriological quality of some chicken meat represented by fresh and frozen thigh and breast through: determination of *Staphylococci* count, isolation and identification of *Staph. aureus* and confirmation of the results by using of polymerase chain reaction (PCR).

## 2. MATERIALS AND METHODS

### 2.1. Collection of samples

100 chicken meat samples were collected from different places (supermarkets, abattoirs, butcher's shops) at Aswan city, Egypt. 50 fresh chicken meat samples (25thigh and 25breast) and 50 frozen chicken meat samples (25thigh and 25breast).

All samples were placed in separate sterile plastic bags to prevent cross contamination and spilling and were immediately transported to the central laboratory; Faculty of Veterinary Medicine; Aswan University in a cooler with ice packs then prepared to take samples for cultivation on a selective media (Harrigan, 1998).

For microbiological analyses, serial dilutions of the collected samples were performed in 0.1% buffered peptone water up to 10<sup>-6</sup>. The collected samples were tested for:

*Staphylococcus aureus* count: The applied technique recommended by ISO 6888-1 (ISO, 2002). Then biochemical identification according to (MacFaddin, 2000) were done.

### 2.2. Confirmation of the results by using of PCR as showed in table (1)

The first denaturation during 5 min at 94°C then following by 30 cycles of denaturation (94°C for 2 min), annealing (50°C for 1 min), and extension (72°C for 1 min). The last extension step (72 °C for 5 min) was done after the finishing of the cycles. The PCR products electrophoresed in 1% agarose gel and stained with ethidium bromide.

## 3. RESULTS AND DISCUSSION

Chicken meats were subjected to many types of contamination. The result of the obtained study revealed that the incidence of isolated *Staph. aureus* from frozen and fresh thigh and breast chicken meat were 40, 36, 32 and 32%; respectively as showed in Table (2). The results of isolation of *Staph. aureus* in frozen chicken breast and thigh were more than that recorded by Abo-Samra., (2013).

These results may be attribute to poor manufacturing practices of food vendor, poor personal hygiene and sometimes duo to storage conditions are improper (Musa and Okande, 2002) or

may due to the withstand high sodium concentration (Jawetz et al., 2008). also, less than that recorded by Islam et al., (2014) and El-sayed (2015).

Table 1: Primer sequences of *Staph. aureus* identification

Target gene	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
<i>sea</i> (F)	5' TTGGAAACGGTTAAAACGAA'3	120	Rall et al. (2008)
<i>sea</i> (R)	5' GAACCTTCCCATCAAAAACA '3		
<i>seb</i> (F)	5' TCGCATCAAACCTGACAAACG '3	478	
<i>seb</i> (R)	5' GCGGTA CTCTATAAGTGCC '3		
<i>sec</i> (F)	5' GACATAAAAGCTAGGAATTT '3	257	
<i>sec</i> (R)	5' AAATCGGATTAACATTATCC '3		
<i>sed</i> (F)	5' CTAGTTTGGTAATATCTCCT '3	317	
<i>sed</i> (R)	5' TAATGCTATATCTTATAGGG '3		

Amplification of *Staph. aureus* enterotoxin genes (Rall et al., 2008)

The results of isolation of *Staph. aureus* in fresh chicken breast and thigh were more than that recorded by Abo-Samra., (2013) and Miranda et al., (2009) and less than that recorded by El-sayed (2015).

The presence of *Staph. aureus* may attribute to contamination that may be directly introduced into the food by workers with skin lesions containing *Staph. aureus* or coughing or sneezing or indirectly through working surfaces and used knives (Yeh et al., 2004).

Table 2: Incidence of *Staph. aureus* contamination in the examined samples of chicken meats

Type of Sample	Type of bacteria	<i>Staph. aureus</i>	
		n. +ve samples	%
Frozen chicken thigh (n=25)		10	40
Frozen chicken breast (n=25)		9	36
Fresh chicken thigh (n=25)		8	32
Fresh chicken breast (n=25)		8	32
Total		35	35

The *Staphylococcal* count in examined fresh and frozen chicken thigh and breast were  $4.11 \times 10^3$ ,  $2.53 \times 10^2$ ,  $7.55 \times 10^2$ ,  $5.8 \times 10^2$ ; respectively **Table (3)**.

The result of isolation of *Staphylococcal* count in fresh chicken meat samples was more than that reported by **Bhandari et al., (2013)**.

The result of isolation of *Staphylococcal* count in frozen chicken meat samples was less than that reported by **Islam et al., (2014)**.

The results of the obtained study revealed that fresh chicken thigh, were positive for *sea* gene of *Staph-aureus* strains, frozen chicken meat samples were positive for *sec* gene of *Staph-aureus* and fresh chicken thigh, frozen chicken breast samples were negative for *sea*, *seb*, *sed* and *sea* genes of *Staph. aureus*. as showed in **Fig.1**.

The results cleared that PCR is an ideal method for identification of foodborne pathogens, as was more effective, more sensitive, less labour, reduces time and effort after using gradient PCR in validation of each microbe (**Armany et al., 2016**).

Table 3: Bacterial count /g of *Staph. aureus* in the examined samples of chicken meats and its products

Type of Sample	Type of bacteria	<i>Staph. aureus</i>			
	N. of +ve samples	Min.	Max.	Mean±SE	
Frozen chicken thigh (n=25)	10	2x10 <sup>2</sup>	2x10 <sup>4</sup>	7.55x10 <sup>2</sup> ±1.99x10 <sup>2</sup>	
Frozen chicken breast (n=25)	9	1x10 <sup>2</sup>	1.5x10 <sup>2</sup>	5.8x10 <sup>2</sup> ±1.14x10 <sup>2</sup>	
Fresh chicken thigh (n=25)	8	9x10	4.3x10 <sup>3</sup>	4.11x10 <sup>3</sup> ±1.05x10 <sup>3</sup>	
Fresh chicken breast (n=25)	8	8x10	1.5x10 <sup>3</sup>	2.53x10 <sup>3</sup> ±0.52x10 <sup>3</sup>	

a.b.c.= significant difference symbols ( $p>0.05$ ) not significant result.



Fig1: Incidence of *Staph. aureus* in chicken meat

Agarose gel electrophoresis of multiplex PCR of *sea* (120 bp), *seb* (478 bp), *sec* (257 bp) and *sed* (317 bp) as enterotoxin genes for characterization of *Staph. aureus*.

Lane 1, 2, 5 (fresh chicken thigh) 3,4,6 (frozen chicken breast).

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

Lane C+: Control positive for *sea*, *seb*, *sec* and *sed* genes.

Lane C-: Control negative.

Lane 4: Positive *Staph. aureus* strains for *sec* gene

Lanes 5: Positive *Staph. aureus* strains for *sea* gene.

Lanes 1, 2, 3& 6: Negative *Staph. aureus* strains for *sea*, *seb*, *sec* and *sed* genes.

## Conclusion

The presented results in this study concluded that the examined fresh and frozen chicken meat samples were highly contaminated with *Staph. aureus* reflecting unhygienic measures and unsuitable environmental conditions during handling, transporting, processing and storage.

Suggestive recommendation points should be followed to prettifying up the microbiological excellence of fresh and frozen meat to prevent growth and multiplication of bacteria that may contaminate and invade them as follow:

- 1- During receiving frozen chicken meat, should be sure that these products has been consistently hard frozen, there are no thick ice crystals on the outer cartons and these cartons are not wet and there are no ice crystals or frost on the inner cartons.
- 2- All vehicles and containers used for transport should be fitted with temperature records to monitor the environment and transfer should be as rapid as possible.

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