

Original research

Incidence of *Staphylococcus aureus* in raw fresh and frozen beef meat in Aswan city, Egypt

Aml Mohamed^{*1}, Mohamed Karmi¹, Mohamed Abdelfattah Maky²

¹Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Aswan University, Aswan, Egypt

²Department of Food Hygiene and Control, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

Received: 11/4/2021

Accepted: 5/5/2021

© Unit of Environmental Studies and Development, Aswan University

Abstract:

Meat is considered an excellent source of high quality animal protein, vitamins as B complex and minerals as iron. Therefore a total of 100 fresh and frozen meat samples (beef) were collected from Aswan city supermarkets for evaluation of the presence of contamination with *Staphylococcus aureus*. The results revealed that the incidence of isolated *Staph. aureus* from frozen meat samples were 36% and from fresh meat samples were 34% and the *Staphylococcal* count in examined fresh and frozen meat were $2.1 \times 10^3 \pm 1.05 \times 10^3$ and $2.2 \times 10^3 \pm 0.52 \times 10^3$; respectively. The results confirmed by using the Polymease Chain Reaction (PCR).

Keywords: *Staphylococcus aureus*, fresh meat, frozen meat, PCR.

1- INTRODUCTION

Meat considered as an important source of protein, fat, vitamins and minerals, low in carbohydrate content. All muscle tissue is high in protein, contain the essential amino acids and is good source of zinc, vitamin B₁₂, selenium, phosphorus, vitamin B₆, riboflavin and iron. Several forms of meat are rich in vitamin K. Muscle tissue is low in carbohydrates and do not contain dietary fiber. But, taste quality is different between meats, the proteins, vitamins and minerals available from meats are generally consistent (Adu-Gyamfi et al., 2012). The main source of meat contamination at slaughter processes as clothing of workers, processing equipments like saws boning, tables and mincers water used to clean carcasses, hands and equipments (Upmann et al., 2000).

Staphylococcus is gram-positive, facultative aerobic and sometimes in encapsulated organism, that commissioned for infection of many tissues and a several diseases. Those bacterium are carried mainly transiently by about 20 and 30% of healthy adults on the skin and nares, respectively (Xu et al., 2008).

Corresponding author*: E-mail address: dr.amlmohamed50@yahoo.com

More than 30 dissident types of *Staphylococci* make infection for peoples and its associated illness can differ from mild to severe form, no processing needed to even potentially fatal. The most of those infections done by *Staph. aureus*, whose has been regarded as leading issues together in medicine and food safety and can typically causes a great differ of infections, as skin infections and occasionally endocarditis, osteomyelitis, pneumonia, scalded skin syndrome, gastroenteritis, and toxic shock syndrome (FDA, 2012).

Most of people infections are come from the consumption of contaminated meat (Chittick et al, 2006) so; the aim of the present research was carried out for evaluation bacteriological quality of some meat samples (beef) though: determination of *staphylococcus aureus* count, isolation and identification of *Staph. aureus* and confirmation of the results using of polymerase chain reaction (PCR).

2- MATERIALS AND METHODS

1.2. Collection of samples

100 samples from raw fresh and frozen meat were collected from different places at Aswan city, Egypt, 50 samples for each.

All samples were placed in separate sterile plastic bags to prevent cross contamination and spilling and were immediately transferred to the central laboratory of the; Faculty of Veterinary Medicine; Aswan University in a suitable cooler with many ice then prepared to take samples for cultivation on a selective media (MacFaddin, 2000).

2.2. Microbiological analysis:

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

Staphylococcus aureus count: The applied technique recommended by ISO 6888-1 (ISO, 2002).

Then biochemical identification was done according to (MacFaddin, 2000).

3.2. Confirmation of the results by using PCR as showed in Table (1)

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' GCGGTACTCTATAAGTGCC '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 first denaturation was 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

Fresh and frozen meats were subjected to many sources of contamination. The result of the obtained study revealed that the incidence of isolated *Staph. aureus* from fresh and frozen meats were 34% and 36%; respectively as showed in **Table (2)** and **Fig (1)**.

The result of isolation of *Staph. aureus* from fresh meat was more than that said by **Jar et al. (2014)** and **Binh et al. (2017)** and nearly similar to that recorded by **Khalalfalla et al. (2017)**.

The result of isolation of *Staph. aureus* from frozen meat was more than that recorded by **Philipis et al. (2001)**.

Finding of *Staph. aureus* in meat refer to poor hygiene of meat handlers and lack of sterilization of utensils **Plaatjies et al. (2004)**.

The conclusion of isolation of *Staphylococcal* count in fresh meat samples was $2.1 \times 10^3 \pm 1.05 \times 10^3$ cfu/g more than that reported by **Shaltout et al. (2016)**. The result of isolation of *Staphylococcal* count in frozen meat samples was $2.2 \times 10^3 \pm 0.52 \times 10^3$ cfu/g. as showed in **Table (3)**.

This result of frozen meat was more than fresh meat may be due to excess handling of frozen meat and leave to more time at surfaces with un clean utensils before freezing.

The conclusion of the present study indicated that frozen meat samples were positive for *sec* gene of *Staph- aureus*, fresh meat and frozen meat samples were negative for *sea*, *seb*, *sed* and *sea* genes of *Staph. aureus*. as showed in **Fig 1**.

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

Table 2: Incidence of bacterial contamination in the examined fresh and frozen meat samples.

Type of bacteria Type of Sample	<i>Staph. aureus</i>	
	n. +ve samples	%
Fresh meat (n=50)	17	34
Frozen meat (n=50)	18	36
Total	35	35

Table (3): Bacterial count /g of *Staph. aureus* (cfu/g) in the examined fresh and frozen meat samples.

Type of bacteria	<i>Staph. aureus</i>			
	N. of +ve samples	Min.	Max.	Mean±SE
Fresh meat (n=50)	17	2x10 ²	2.2x10 ⁴	2.1x10 ³ ±1.05x10 ³
Frozen meat (n=50)	18	1x10 ²	1.5x10 ⁴	2.2x10 ³ ±0.52x10 ³

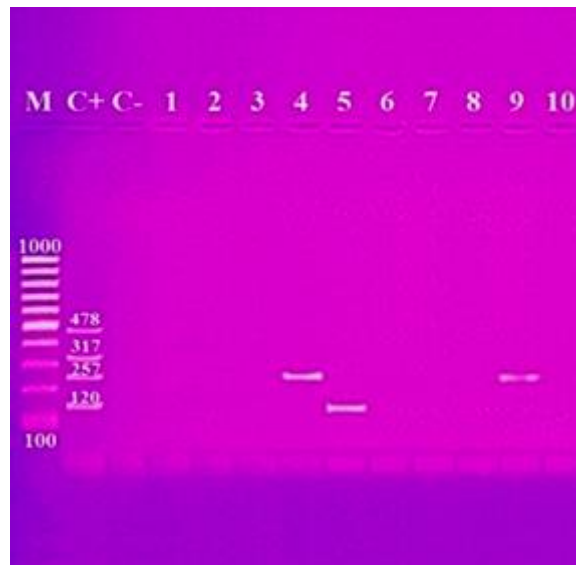


Fig. (1): Incidence of *Staph. aureus* in fresh and frozen meat

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

Lanes 1, 2,3,4,5 (fresh meat) 6, 7, 8,9,10 (frozen meat)

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

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

Lane C-: Control negative.

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

Lanes 4 & 9: Positive *S. aureus* strains for *sec* gene.

Lanes 1, 2, 3, 6, 7, 8, 10: Negative *S. aureus* strains for *sea*, *seb*, *sec* and *sed* genes.

4. Conclusion

The presented results in this study concluded that the examined fresh and frozen 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- The Egyptian government must sent veterinary doctors to the slaughter houses in order to follow up the anti-mortem inspection, slaughtering, post-mortem inspection, packing and loading.
- 2- High hygienic precaution must be recommended with no delay during loading and dis-loading of frozen and fresh meat.

References

- Adu-Gyamfi, A., Torgby-Tetteh, W. and Appiah, V. (2012). Microbiological quality of chicken sold in Accra and determination of D10value of *E. coli*. Food Nutr. Sci. 3 (5): 693-698.
- Armany, A.; Hemmat, M.; Reham, A. and Hanaa, A. (2016). Detection of some foodborne pathogens in meat products by PCR. Benha Vet. Med. J. 30(1): 233 -330.
- Binh, D., Minh, N. and Douan, T. (2017). Prevalence of *L. monocytogenes*, *E. coli*, *Salmonella Spp.* and *Staph. aureus* bacteria contamination on meat at public market in the North of Vietnam. J Microbiol Infect Dis. 5(5): 1-22.
- Chittick, P., Sulka, A., Tauxe, R. and Fry, A. (2006). A summary of national reports of foodborne outbreaks of Salmonella Heidelberg infections in the United States: clues for disease prevention. Journal of Food Protection, 69, 1150–1153.
- FDA (Food and Drug Administration) (2012). Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed. US Food and Drug Administration, Silver Spring.
- Harrigan, W. (1998). Laboratory method in food microbiology. 3rd. Academic Press, San Diego, Calif. Pp. 43-73.
- ISO (International organization for standardization, 6888-1) (2002): Microbiology of Food and Animal Feeding Stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – part 1: technique using Baird-Parker agar medium. ISO, Geneva, Switzerland.
- Jar, A., Seaab, I. and Kalid, Y. (2014). Isolation and identification of some pathogenic bacterial species contaminated from meats in butchers shops and kebab restaurants in AL-Kut city. Euphrates J. of Agriculture Science. 6 (4): 30-37.
- Khalalfalla F. A., Fatma H. M. Ali and Saif-Alnasr M.M. (2017): Microbiological quality of retail meats. Vet. Med. Res. Vol., 24(2): 311-321.
- MacFaddin, J. F. (2000): Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.
- Phillips, D.; Summer, J.; Alexander, J. and Dutton, K. (2001). Microbiological quality of Austerillian beef. J. Food Protec. 64(5): 692-696.
- Plaatjies, Z.; Lues, J. and Buys, E. (2004). *Staphylococcal* growth in fresh vacuum-packed red meat at various storage conditions. 8th World Congress on Environmental Health. Durban, South Africa.

- Rall, V., Vieira, F., Rall, R., Vieitis, R., Fernandes, A., Candeias, J., Cardoso, K. and Araujo, J. (2008): PCR detection of staphylococcal enterotoxin genes in *Staph. aureus* strains isolated from raw and pasteurized milk. *Vet. Microbiol.* 132: 408–413
- Shaltout, F.; Salem, M.; Khater, F. and Lela, A. (2016). Studies on bacteriological profile of some meat and meat products. *Benha Vet Med J.* 31 (2): 213-219.
- Upmann, M., Jakob, P. and Reuter, G. (2000). Microbial transfer during cutting of pork. *Dairy food and Environmental sanitation J.* 20: 14-23.
- Xu, Z., Li, L., Alam, M., Yamasaki, S. and Shi, L. (2008). First confirmation of integron-bearing methicillin-resistant *Staph. aureus*. *Curr. Microbiol. J.* 57: 264-268.