Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Molecular detection of enterotoxigenic *Staphylococcus aureus* in some ready to eat meat-based sandwiches

Rehab Gaafar[1], Faten Hasanine[2], Fahim Shaltout[2], Marionette Zaghloul[1]

[1]*Department of Food Hygiene, Animal Health Research Institute, Benha branch*

[2]*Department of Food Hygiene, Faculty of Veterinary Medicine, Benha University*

ARTICLE INFO

Keywords

Meat products
Multiples PCR
RTE sandwiches
Staphylococci
S. aureus enterotoxins

Received 04/08/2019
Accepted 30/10/2019
Available On-Line 12/05/2020

ABSTRACT

Ready-to-eat (RTE) meat products sandwiches are one of the most popular fast foods on which consumers depend. Unfortunately, it may be exposed to many contamination sources and harbored many food poisonings factors, especially with street vendors emerging in the developing world. *Staphylococcus aureus* (*S. aureus*) and its enterotoxins (SEs) are one of the most recorded food intoxications causes which contribute a health hazard risk. Therefore, one-hundred and twenty samples of beef kofta, bovine liver, chicken nuggets, and fish fillet RTE sandwiches (30 of each) were collected randomly from different street vendors and restaurants at Benha city, Qalubiya Governorate, Egypt, for bacteriological and molecular detection of coagulase-positive enterotoxigenic *S. aureus* contamination. Results revealed that beef kofta sandwich samples recorded the highest incidences and mean counts of *S. aureus* detection 40% and 12×10^3 CFU/g, respectively; followed by chicken nuggets, fish fillet, and bovine liver sandwich samples. Antimicrobial sensitivity on the isolated strains revealed high resistance to nalidixic acid, while mostly sensitive to erythromycin. On the other hand, molecular detection of SEs genes revealed detection of *SeA*, *SeC*, and *SeD* genes carrying strains where *SeA* was the most frequently detected. Mixed strain carrying both *SeC* and *SeD* was also detected. *SeB* gene was failed to be detect in any of examined isolates. It is obvious that RTE meat product sandwiches may pose a risk to consumer's health and encourage the authorities to exert more control over street vendors and fast food restaurants.

1. INTRODUCTION

Staphylococci are Gram-positive bacteria arranged in grape-like clusters cocci, facultative anaerobe, non-spore, and non-motile bacteria. *Staphylococcus aureus* (*S. aureus*) is considered the virulent food poisoning species among members of the Staphylococcus species (Liu et al., 2005). These bacteria are used as an indicator of hygienic faults during food production, preparation, serving and improper thermal processing (Alexandra et al., 2011).

Staphylococcus aureus is considered as community-acquired pathogens, USFDA (2012) reported that *S. aureus* may be found in foods, dust, air, and sewage, or on food equipment, and food preparation surfaces. Staphylococci are present in throats and nasal passages, on hair and skin of more than 50% of apparently healthy individuals. However, food handlers are usually the main source of food contamination. Equipment and environmental surfaces also can be sources of contamination with *S. aureus*.

Schelin et al. (2011) found that staphylococcal food poisoning (SFP) is a foodborne intoxication caused by the consumption of *S. aureus* toxins contaminated foods that have been improperly prepared or stored. The severity of the illness is related mainly to the concentration of toxin, and the health status of the person. SFP can be caused by as little as 20-100 ng of *S. aureus* heat-stable enterotoxins. The enterotoxins production and onset of food poisoning are correlated with bacterial growth, which means, the more bacterial growth, the more toxin production. Therefore, *S. aureus* count is usually indicating the wholesome of the food product and its safety for human consumption.

Staphylococcus aureus enterotoxins (SEs) are the major virulence factor causing food poisoning. The main *SEs* incriminated in SFP are staphylococcal enterotoxin A (*SEA*), staphylococcal enterotoxin B (*SEB*), staphylococcal enterotoxins C (*SEC*), and staphylococcal enterotoxins D (*SED*). SFP signs are characterized by rapid onset including nausea and violent vomiting with or without diarrhea. *S. aureus* enterotoxin type A is the most common cause of SFP worldwide, but the involvement of other classical SEs (*SEB* to *SEE*) have been also recorded (Chiang et al., 2008; Argudin et al., 2010).

* Corresponding author **Rehab Gaafar.** Department of Food Hygiene, Animal Health Research Institute, Benha branch

Antimicrobial resistance is a global public health concern and the prevalence of antimicrobial resistance among food-borne pathogens increased during recent decades (Akbar and anal 2013).

Therefore, this study aimed to detect the incidence and antimicrobial sensitivity of *S. aureus* contamination in some RTE meat products sandwiches, and molecular detection of enterotoxigenic strains as well.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A grand total of 120 random samples of ready to eat meat products sandwiches represented by (beef kofta, bovine liver, fish fillet, and chicken nuggets), 30 of each, were collected from restaurants and street vendors at Benha city, Qalubiya governorate, Egypt. Each sample was kept in a separate sterile plastic bag and put in an icebox then transferred to the laboratory under complete aseptic conditions without undue delay and examined bacteriologically to detection the incidence of *S. aureus*, and molecular detection of enterotoxigenic strains by multiplex PCR.

2.2. Preparation of samples according to (APHA, 2013):

Twenty-five grams of the sample were mixed with 225 ml sterile 0.1% peptone water, the contents were homogenized at Stomacher (M A 106402, France, 450 to 640 strokes per minute) for 2 minutes, 1 ml of the mixture was transferred into separate tube each containing 9 ml sterile 0.1% peptone water, from which tenth- fold serial dilutions were prepared. The prepared samples were subjected to the following bacteriological examination:

2.3. Determination of S. aureus.

2.3.1. Counting and isolation of S. aureus according to (ISO 6888-1:1999, A1:2003).

0.1 ml from the previously prepared serial dilution was spread over Baird-Parker agar plates and incubated at 35±2 °C for 24-48 hours. Suspected colonies were counted and isolated for more identification.

2.3.2. Identification of Staphylococcus aureus.

Morphological examination by Gram's staining (Cruickshank et al., 1975).

2.3.3. Biochemical identification (MacFaddin, 2000).

2.3.4. Coagulase test (APHA, 1992):

0.1 ml of BHI broth cultured with S. aureus isolate was transferred to Wassermann tubes containing 0.3 ml of sterile reconstituted rabbit plasma. Inoculated tubes were incubated at 37 °C for 4 hours. The tubes were reexamined for clotting (fibrin clot formation). The extent of coagulase reaction was recorded. Tubes were left at room temperature for an additional 20 hours and then re-examined for clot formation. The extent of coagulation of the plasma was recorded after 4 and 24 hours. S. aureus is coagulase positive.

2.4. In-Vitro anti-microbial sensitivity test for isolated *S. aureus* was performed according to (Deresse et al. 2012) by disc-diffusion technique.

2.5. Molecular detection of S. aureus enterotoxin genes:

Ten isolates of each confirmed coagulase positive *S. aureus* strains were sent to the Central Laboratory for Food Analysis, Faculty of Veterinary Medicine, Benha University, Egypt; and molecularly examined for presence of *S. aureus* carrying enterotoxins genes using multiplex PCR.

- Primer sequences of S. aureus used for PCR system following Rall et al. (2008): Different enterotoxin primers (Pharmacia Biotech) specific for demonstration of SEs (A, B, C, and D) as virulence genes were applied as shown in the following table.

Primer sequences of S. aureus used for PCR system

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Ref.
SEA (F)	5′ TTGGAAACGGTTAAAACGAA3'	120	Rall et al.
SEA (R)	5′ GAACCTTCCCATCAAAAACA 3'	120	(2008)
SEB (F)	5′ TCGCATCAAACTGACAAACG 3'	478	
SEB (R)	5′ GCGGTACTCTATAAGTGCC 3'	478	
SEC (F)	5′ GACATAAAAGCTAGGAATTT 3'	257	
SEC (R)	5′ AAATCGGATTAACATTATCC 3'	257	
SED (F)	5′ CTAGTTTGGTAATATCTCCT 3'	317	
SED (R)	5′ TAATGCTATATCTTATAGGG 3'	317	

- DNA Extraction using QIA amp kit (Shah et al., 2009).
- Amplification of SEs genes (Rall et al., 2008).
- Final amplified product was inoculated and run on agarose gel electrophoresis and interpreted under UV reader.

2.6. Statistical Analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

Recorded results in table (1) shows that *S. aureus* could be detected in 41(34.2%) of total examined samples, with incidences of 40.0, 36.6, 33.3, and 26.6%; with mean values 12×10^3, 9.2×10^3, 8.9×10^3, and 7.1×10^3 CFU/g for beef kofta, chicken nuggets, fish fillet, and bovine liver samples, respectively. Statistical analysis revealed significant ($P \leq 0.05$) difference between samples where kofta samples were the most contaminated followed by chicken nuggets, fish fillet, but bovine liver samples were the lowest contaminated samples with *S. aureus*.

Table 1 Statistical analytical results of Staphylococcus aureus count (cfu/g) in the examined sandwiches samples (n=30)

Products	+ ve Samples		Min.	Max.	Mean± S.E.
	No.	%			
~~Products~~			~~Min.~~	~~Max.~~	~~Mean± S.E.~~
Beef kofta	12	40.0*	8.2×10^3	1.9×10^4	$12\pm0.78\times10^{3a}$
Beef liver	8	26.6*	1.2×10^3	1.3×10^4	$7.1\pm0.95\times10^{3c}$
Fish fillet	10	33.3*	6.9×10^3	1.1×10^4	$8.9\pm0.36\times10^{3bc}$
Chicken nuggets	11	36.6*	5.2×10^3	1.4×10^4	$9.2\pm0.65\times10^{3b}$
Total	41	34.2**			

Values within a column with different superscript letters were significantly different at ($P \leq 0.05$). * Percentage in relation to total number of each sample (30). ** Percentage in relation to total number of samples (120).

Prevalence of antimicrobial sensitivity of isolated *S. aureus* strains as presented in table (2) revealed variable sensitivities and resistance to different used antimicrobials, where most of strains were sensitive to erythromycin (70.7%) followed by norfloxacin (65.8%), and ampicillin (56.1%), while all isolates were resistant to Nalidixic acid (100%).

Table 2 Percentages of Antimicrobial susceptibility of *S. aureus* species Isolated from the examined meat products samples (n=41).

Antimicrobial agent	S		I		R	
Antimicrobial agent	No.	%	No.	%	No.	%
Nalidixic acid (NA)	-	-	-	-	41	100
Sulphamethoxazol (SXT)	-	-	3	7.3	38	92.7
Kanamycin (K)	3	7.3	-	-	38	92.7
Neomycin (N)	3	7.3	9	21.9	29	70.7
Gentamicin (G)	6	14.6	9	21.9	26	63.4
Oxytetracycline (T)	6	14.6	12	29.2	23	56.1
Penicillin (P)	9	21.9	15	36.5	17	41.4
Chloramphenicol (C)	12	29.2	15	36.5	14	34.1
Amoxicillin(AMX)	18	43.9	14	34.1	9	21.9
Streptomycin (S)	19	46.3	5	12.2	17	41.5
Ciprofloxacin (CP)	15	36.6	4	9.75	22	53.6
Ampicillin (AM)	23	56.1	7	17.1	11	26.8
Norfloxacin (NOR)	27	65.8	5	12.2	9	21.9
Erythromycin (E)	29	70.7	7	17.1	5	12.2

S: Sensitive. I: Intermediate. R: Resistant

For further confirmatory detection of enterotoxigenic *S. aureus*, multiplex PCR detection of SEs genes was conducted as shown in figure (1), where examined strains gave molecular bands at 120, 257, and 317 pb which indicates containing *S. aureus* enterotoxin genes types A, C, and D, respectively. Results revealed that SE type A gene was the most frequently detected. Results showed detection of mixed strains carrying both SE of type C and D, while failed to detect SE type B gene.

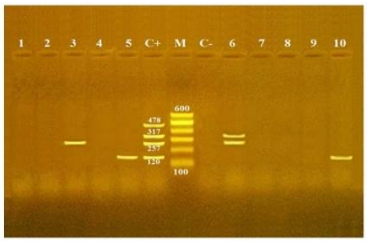

Fig 1 Agarose gel electrophoresis of multiplex PCR of SEA (120 bp), SEB (478 bp), SEC (257 bp) and SED (317 bp) enterotoxin genes for characterization of *S. aureus*. Lan M: 100 bp ladder as molecular size DNA marker. Lan C+: Control positive for SEA, SEB, SEC and SED genes. Lan C-: Control negative. Lan 3: Positive *S. aureus* strains for SEA. Lan 5& 10: Positive *S. aureus* strains for SEA. Lan 6: Positive *S. aureus* for SEC and SED genes. Lan 1, 2, 4, 7, 7, 8 and 9: Negative *S. aureus* for enterotoxins.

4. DISCUSSION

Microbiological quality of ready to eat meat sandwiches depends mainly on the initial quality of raw materials and ingredients used in preparation, cooking process, and sanitary practices for personnel and for cooking/processing utensils; even though some ingredients reach a temperature that is ideal to ensure that the food is cooked thoroughly, cross-contamination during preparation may be occurred due to the use of fresh non-heat-treated vegetables and unhygienic handling (Kayaardi et al., 2006).

From the point of food hygiene view, *S. aureus* has been recorded as one of the most virulent and reported as food poisoning outbreak pathogens; counts and incidences of *S. aureus* in examined samples that tabulated in table (1) were somewhat agreed with results recorded by El-kewaiey (2012) ($6.0x10^3$ CFU\g for nuggets samples); Angaw et al. (2015) ($6.4x10^3$ CFU\g for fried fish samples); Elshazly (2015) ($3.6x10^4$ CFU\g for kofta sandwich samples), and Abdu-Elaziz (2018) ($1.1x10^3$ CFU\g for liver sandwiches); while they were lower than those recorded by Rezk (2017) ($1.04x10^4$ CFU\g of examined liver samples); Bagumire and Karumuna (2017) ($6.6x10^4$ CFU\g for cooked chicken meat product sandwiches); (morshdy et al., 2018; and Rawash, 2015) (detected *S. aureus* in 90, and 60% of examined RTE kofta samples); in addition, results were higher than those recorded by Abd-El-Malek (2014) ($6.0x10^2$ CFU\g of examined liver sandwiches); Hassanien et al. (2014) (14.28% of RTE fish samples); Contreras et al. (2015) (found *S. aureus* in 8% of examined chicken sandwiches); Mashak et al. (2015) ($1.4x10^2$ CFU\g for chicken meat meals); Wu et al. (2018) (9.8x10 and $1.5x10^2$ CFU\g for RTE poultry and beef meals), while disagreed with Cakli et al. (2005) (failed to detect *S. aureus* in fish fillet samples).

The variation in the results between different authors may be due to the differences in manufacture practices, handling from producers to consumers and the effectiveness of hygienic measures applied during production. The presence of such pathogenic bacteria in food is considered as an indicator of faults during preparation, handling, storage or service which may come through the used raw food, food handlers and the surrounding environment. Generally, the high bacterial counts of examined meat products may be due to contamination of raw materials used for processing of these products; however, spices, equipment, dressings, knives, and other additives are considered as the source of contamination of meat during preparation.

The recent results proved that the examined kofta sandwich samples were the most contaminated samples, followed by chicken nuggets, fish fillet, and liver samples which may be due to the fact that longer time of heat treatment in case of frying has a more lethal effect on the bacterial contaminations.

The emergence of antibiotic resistance in bacteria that may be encouraged by extra use of antibiotics in animal farm can make treatment of human infections more difficult, so it has been recommended that antibiotics used for animal diseases treatment and control should not be used for human treatment (Darwish et al., 2013).

Results of antimicrobial sensitivity of *S. aureus* isolates that summarized in table (2) were somewhat agreed with those recorded by Ibrahim (2016), and Hosny (2016) who recorded a multidrug resistance of their *S. aureus* strains that were isolated from meat and meat products.

Staphylococcal food poisoning (SFP) is one of the most prevalent causes of gastroenteritis worldwide that rose after the consumption of contaminated foods with enterotoxigenic *S. aureus* and produced

enterotoxins. *S. aureus* enterotoxins (*SEA* to *SEE*) are recognized as to be responsible for 95% of staphylococcal food poisoning cases and *SEA* and *SED* are most common enterotoxins recovered from food poisoning outbreaks. Staphylococcal enterotoxins are considered a potential biological threat because of their stability at high temperature (100°C for 1hr.) and their ability to incapacitate individuals for several days to two weeks (De Buyser et al., 2001; Bhatia and Zahoor, 2007).

Molecular detection of *S. aureus* enterotoxin genes using multiplex PCR was in agreement with Argudin *et al.* (2010); Balaban and Rasooly (2000) who recorded that SEs produced by some strains of *S. aureus* are the causative agents of SFD, and SeA is the most common toxin implicated in such events. *SEA* was recovered from 77.8% of all SFD outbreaks in the United States followed by *SED* (37.5%) and *SEB* (10%); *SEA* is the most commonly found enterotoxin among SFD outbreaks in Japan, France, and UK. However, *SEC* and *SEE* are also implicated with SFD. Results of detection of enterotoxigenic *S. aureus* strains were in agreement with Rezk (2017) and Naguib (2017) who could detect enterotoxigenic *S. aureus* in RTE meat products.

5. CONCULOSIONS

Our results show that the examined RTE kofta sandwich samples were the most contaminated with *S. aureus*; in addition, isolated strains were confirmed to carry *SEA*, *SEC*, and *SED* genes, which indicating that most of RTE sandwiches preparing facilities in Benha city are neglecting the hygienic measures during processing and handling of ready to eat meat products which render it as a potential source of health hazard, so strict control and supervision should be applied by responsible authorities to overcome potential food poisoning.

6. REFERENCES

1. Abd-El-Malek, A.M. 2014. Microbiological quality of ready-to-eat liver sandwiches (Kibda). Global Veterinaria, 13(6): 1097-1102.
2. Abdu-Elaziz, Y.A.A. 2018. Microbiological quality of ready to eat meat meals at Ismailia City. Thesis, Master of Veterinary Medicine, Suez Canal University, Egypt.
3. Akbar, A., Anal, A.K. 2013. Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat. Asian Pacific Journal of Tropical Biomedicine, 3(2):163-168.
4. Alexandra, F., Britta, K., Gladys, K., Beatriz, G.R., Katja, A., Hammerl, J.A., Annemarie, K., Juliane, B., Bernd, A., Bernd-Alois, T. 2011. Methicillin susceptible and resistant *Staphylococcus aureus* from farm to fork impact on food safety. Tehnologija Mesa, 52 (1): 60-65.
5. American Public Health Association "APHA" 2013. Compendium of methods for the microbiological examination of food. T. Matthew Taylor, John N. Sofos, Peter Bodnaruk, and Gary R. Acuff (Eds.), 4th Ed., Ch. 2, Washington DC., USA.
6. Angaw, M., Kibret, M., Abera, B. 2015. Microbiological quality and safety of sambusa and fried fish in Bahir Dar, Ethiopia. Food Science and Quality Management, 40: 96-108.
7. Argudin, M.A., Mendoza, M.C., Rodicio, M.R. 2010. Food poisoning and *S. aureus* enterotoxins. Toxins J., 2(7):1751-1773.
8. Bagumire, A., Karumuna, R. 2017. Bacterial contamination of ready-to-eat meats vended in highway markets in Uganda. African Journal of Food Science, 11(6): 160-170.
9. Balaban, N., Rasooly, A. 2000. Staphylococcal enterotoxins. International Journal of Food Microbiology, 61(1):1-10.
10. Bhatia, A., Zahoor, S. 2007. Staphylococcus enterotoxins: A review. J. Clinical and Diagnostic Research, 1:188-197.
11. Cakli, S.K., Taskaya, L., Kisla, D., Ufuk, E., Ataman, C.A., Cadun, A., Kilinc, B., Maleki. R.H. 2005. Production and quality of fish fingers from different fish species. European Food Research Technology, 220: 526–530.
12. Chiang, Y.C., Liao, W.W., Fan, C.M., Pai, W.Y., Chiou, C.S., Tsen, H.Y. 2008. PCR detection of staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in *Staphylococcus aureus* isolates from food-poisoning cases in Taiwan. International J. Food Microbiology, 121(l): 66-73.
13. Contreras, C.P.A., da Silva, L.N.N., Ferreira, D.C.G., Ferreira, J.D.S., Almeida, R.C. 2015. Prevalence of methicillin-resistant *Staphylococcus aureus* in raw hamburgers and ready-to-eat sandwiches commercialized in supermarkets and fast food outlets in Brazil. Food and Nutrition Sciences, 6: 1324-1331.
14. Cruickshank, R.; Duguid, J.; Marmion, B. and Swain, R.H. 1975. Medical Microbiology. 12th Ed., Edinburg, London and New York.
15. Darwish, W.S., Eldaly, E.A., El-Abbasy, M.T., Ikenaka, Y., Nakayama, S., Ishizuka, M. 2013. Antibiotic residues in food: the African scenario. Japanese Journal of Veterinary Research, 61: 13-22.
16. De Buyser, M.L., Dufour, B., Maire, M., Lafarge, V. 2001. Implication of milk and milk products in food-borne diseases in France and in different industrialized countries. International J. Food Microbiology, 67(1-2): 1-17.
17. Deresse, D., Solomon, G., Dawit, Y. 2012. Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. Annual Clinical Microbiology and Antimicrobials, 11: 26-34.
18. El-Kewaiey, I.A. 2012. Quality assessment of some ready-to-eat and locally produced chicken meat products. Assiut Veterinary Medicine Journal, 58: 132-151.
19. Elshazly, A. 2018. Microbiological quality of meat-based sandwiches at fast food restaurants. Thesis, Master of Veterinary Medicine, Benha University, Egypt.
20. Feldman, D., Ganon, J., Haffman, R., Simpson, J. 2003. The solution for data analysis and presentation graphics. 2nd Ed., Abacus Lancripts, Inc., Berkeley, USA.
21. Hassanien, F.S, Maarouf, A.A., Helmy, N.A. 2014. Food Poisoning microorganisms in fried seafood. Benha Veterinary Medical Journal, 27(2): 116-125.
22. Hosny, D.M.A. 2016. Characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from food products of poultry origin in Egypt. Thesis, Master of Veterinary Medicine, Benha University, Egypt.
23. Ibrahim, M.A. 2016. The proportion of the presence of resistant strains of *S. aureus* isolated from some meat products to antibiotics. Thesis, Master of Veterinary Medicine, Benha University, Egypt.
24. International Organization for Standardization "ISO" 1999. International Organization for Standardization. No. 6888-1:1999, A1:2003. 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 (includes amendment A1:2003).
25. Kayaardi, S., Kayacier, Q.A., Gok, V. 2006. Sensory and chemical analysis of Doner Kebab made from Turkey meat. J. Muscle Food, 17:165-173.

26. Liu, G.Y., Essex, A., Buchanan, J.T., Datta, V., Hoffman, H.M., Bastian, J.F., Fierer, J., Nizet, V. 2005. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. Journal of Experimental Medicine, 202(2): 209-215.
27. MacFaddin, J. F. 2000. Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.
28. Mashak, Z., Langroodi, A.M., Ehsani, A., Fathabad, A.E., Ilkhanipoor, A. 2015. Microbiological quality of ready-to-eat foods of Tehran province. African Journal of Food Science, 9(5): 257-261.
29. Morshdy, A.M., Hussein, M.A., Tharwat, A.E., Fakhry, B.A. 2018. Prevalence of enterotoxigenic and multi-drug-resistant *Staphylococcus aureus* in ready to eat meat sandwiches. Slov. Veterinary Research Journal, 55(20): 367-374.
30. Naguib, R.A. 2017. Detection of virulent genes responsible for Staphylococcus aureus enterotoxins production in chicken meat using PCR. Thesis, PhD of Veterinary Medicine, Benha University, Egypt.
31. Rall, V., Vieira, F., Rall, R., Vieitis, R., Fernandes, A., Candeias, J., Cardoso, K., Araujo, J. 2008. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. Veterinary Microbiology, 132:408-413.
32. Rawash, R. 2015. Bacteriological status of some ready to eat meat and poultry meat meals in Benha city. Thesis, Master of Veterinary Medicine, Benha University, Egypt.
33. Rezk, M.A.E. 2017. Toxigenic Staphylococcus aureus in ready to eat foods in Sharkia. Thesis, PhD. Of Veterinary Medicine, Zagazig University, Egypt.
34. Schelin, J., Carlquist, N.W., Cohn, M.T., Lindqvist, R., Barker, G.C. 2011. The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. Virulence, 2(6):580-592.
35. Shah, D., Shringi, S., Besser, T., Call, D. 2009. Molecular detection of foodborne pathogens, Boca Raton: CRC Press, In: Liu, D. (Ed), Taylor & Francis group, Florida, USA, Pp. 369-389.
36. U.S. Food and Drug Administration "USFDA" 2012. Bad Bug Book: Foodborne pathogenic micro-organisms and natural toxins handbook- *S. aureus*. 10903 New Hampshire Avenue Silver Spring, MD 20993.
37. Wu, S., Huang, J., Wu, Q., Zhang, J., Zhang, F., Yang, X., Wu, H., Zeng, H., Chen, M., Ding, Y., Wang, J., Lei, T., Zhang, S., Xue, L. 2018. Staphylococcus aureus isolated from retail meat and meat products in China: incidence, antibiotic resistance and genetic diversity. Frontiers in Microbiology, 9: 1-14.
38. American Public Health Assoication "APHA" 1992. Compendium of methods for the microbiological examination of foods. 3rd Ed. Speck, H.L. (ed.).Washington D.C. APHA.