Global Veterinaria 11 (3): 335-341, 2013
ISSN 1992-6197
© IDOSI Publications, 2013
DOI: 10.5829/idosi.gv.2013.11.3.1140

Rapid Method for Detection of *Staphylococcus aureus* Enterotoxins in Food

[1]J. El-Jakee, [1]S.A. Marouf, [2]Nagwa S. Ata, [3]Eman H. Abdel-Rahman,
[2,4]Sherein I. Abd El-Moez, [2]A.A. Samy and [2]Walaa E. El-Sayed

[1]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[2]Department of Microbiology and Immunology, Veterinary Division,
National Research Center, Dokki, Giza Egypt
[3]Department of Parasitology and Animal Diseases, Veterinary Division,
National Research Center, Dokki, Giza Egypt
[4]Food Risk Analysis Group, Centre of Excellence and Advanced Science,
National Research Center, Dokki, Giza Egypt

Abstract: Food-borne bacteria have the most concern in public health and food safety. *S. aureus* food poisoning is one of the most economically important food-borne pathogen worldwide. In the present study a total of 250 food samples (milk samples n=50, white soft cheese samples n=50, yoghurt samples n=50, meat and meat products n=50 and chicken products n=50) were investigated bacteriologically to detect the occurrence of enterotoxigenic *S. aureus* among the examined food samples. Out of 250 examined food samples, 127 isolates were identified as *Staphylococcus* species (50.8 %). 32 *S. aureus* isolates were identified from the examined samples with an incidence of 12.8%. The highest isolation rate was observed in raw milk samples (56%) followed by yoghurt samples (22%), chicken products (6%), white soft cheese samples and pasteurized milk samples (4% each) then meat and meat products samples (2%). Using PCR, out of 32 *S. aureus* isolated from the examined food samples 10 isolates could produce enterotoxins. Protein profile analysis of 8 enterotoxigenic *S. aureus* strains were analyzed by Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE). All enterotoxigenic isolates had a band at 26 to 29 kDa. Direct detection of *S. aureus* enterotoxins were investigated among 6 samples (3 raw milk samples, 2 yoghurt samples and one chicken product sample) using mPCR. It could be concluded that *S. aureus* producing enterotoxins present in the food samples.

Key words: Food · PCR · SDS-PAGE · *S. aureus* · *Staphylococci enterotoxins* SEs)

INTRODUCTION

Staphylococcus aureus is a leading cause of food poisoning resulting from the consumption of contaminated food with staphylococcal enterotoxins. Different food can act as a good medium for *S. aureus* such as raw meat and meat products [1], raw milk, dairy products and ready-to-eat foods [2]. Enterotoxins are highly thermostable; normal cooking and pasteurization cannot totally inactivate them, so they cause food poisoning [3]. The onset of symptoms depends on the amount of enterotoxin ingested. Classic SEs antigens have been identified as SEA, SEB, SEC1, SEC2, SEC3, SED and SEE [4]. Recently, several other toxins were detected. RPLA can identify enterotoxins using specific antibodies for each of the enterotoxins, but cross reaction between SEA and SEE have been reported [5]. The enterotoxin genes are accessory genetic elements in *S. aureus*; they are encoded by mobile genes [6]. Specific PCR for detection of enterotoxin genes in food has been developed [7]. The present work was planned to investigate the prevalence of *S. aureus* in food samples and characterization of enterotoxigenic *S. aureus* isolates using multiplex PCR and SDS-PAGE.

Corresponding Author: A.A. Samy, Department of Microbiology and Immunology, Veterinary Division,
National Research Center, Dokki, Giza Egypt.

MATERIALS AND METHODS

Sample Collection: A total of 250 food samples were collected from supermarkets and butchers in Giza and Cairo Governorates, Egypt and investigated bacteriologically for staphylococci infection. The samples were transferred immediately after sampling in cooling ice box to the lab.

Milk Samples: Raw milk samples (n=25) and pasteurized milk samples (n=25) were investigated. About 15 ml of each milk sample was transferred to sterile screw capped tube. The samples were incubated at 37°C for 24 hours then the milk samples were centrifuged at 3000 rpm for 20 minutes before bacteriological cultivation from the sediment.

White Soft Cheese Samples (n=50): Samples of cheese (125g) were randomly collected from dairy markets. Sample homogenate was prepared by homogenizing 10g from each cheese sample separately in 90 ml of nutrient broth for 1.5 min using stomacher in sterile polyethylene bags then 15 ml of each sample were transferred to sterile screw capped tube. The samples were incubated at 37°C for 24 hours.

Yoghurt Samples (n=50): Samples of yoghurt were collected in their containers from dairy markets. Sample homogenate was prepared by homogenizing 10g from each yoghurt sample separately in 90 ml of nutrient broth for 1.5 min using stomacher in sterile polyethylene bags then 15 ml of each sample were transferred to sterile screw capped tube. The samples were incubated at 37°C for 24 hours and then cultivated.

Meat and Meat Products (n=50) and Chicken Product Samples (n=50): Sample homogenate was prepared by homogenizing 10g from each sample separately in 90 ml of nutrient broth for 1.5 min using stomacher in sterile polyethylene bags then 15 ml of each sample were transferred to sterile screw capped tube. The samples were incubated at 37°C for 24 hours and then cultivated.

Isolation and Identification of Staphylococci: The prepared incubated samples were streaked on the surface of mannitol salt agar (Himedia). The inoculated plates were incubated for 24-48 hours at 37°C after which they were examined for colony characters, cellular morphology and the purity of the culture. The colonies were picked up and propagated on Baird-Parker agar (Oxoid). The biochemical and cultural characters of the suspected colonies were studied according to Quinn *et al.* [8].

Detection of Staphylococcal Enterotoxins Genes Using Multiplex Polymerase Chain Reaction: *S. aureus* strains were incubated in 5 ml of brain heart infusion broth (Oxoid) and incubated aerobically at 37°C overnight. About 1.5 ml of the culture was centrifuged at 6000 rpm for 2 minutes and supernatant was discarded. The DNA was extracted from the bacterial pellet according to Sambrook *et al.* [9]. PCR amplifications were conducted according to Pinto *et al.* [10] using sets of Oligonucleotide primers (Table 1) and compounds supplied from Amers Co. Cleveland, Ohio, USA and Advanced Bio-Enzymes Itd. UK. PCR products run on a 1.5% agarose gel stained with 0.5 µg/ml of ethidium bromide electrophoresis was carried out in 1xTAE at 80v for 90 minutes and transferred to transilluminator and photographed.

Table 1: *S. aureus* gene-specific oligonucleotide primers.

Gene	Primer	Oligonucleotide sequence (5'-3')	Product size (bp)	Reference
sea	GSEAR-1	CCTTTGGAAACGGTTAAAACG	127	Becker *et al.* [11]
	GSEAR-2	TCTGAACCTTCCCATCAAAAAC		
seb	GSEBR-1	TCGCATCAAACTGACAAACG	478	Johnson *et al.* [12]
	GSEBR-2	GCAGGTACTCTATAAGTGCC		
sec	GSECR-1	GAACTAGACATAAAAGCTAGG	244	Pinto *et al.* [10]
	GSECR-2	CATTCTTTGTTGTAAGGTGG		
sed	GSEDR-1	CTAGTTTGGTAATATCTCCT	317	Johnson *et al.* [12]
	GSEDR-2	TAATGCTATATCTTATAGGG		
see	GSEER-1	TAGATAAAGTTAAAAAACAAGC	170	Johnson *et al.* [12]
	GSEER-2	TAACTTACCGTGGACCCTTC		

Detection of Enterotoxins of *S. aureus* Isolates by Sodium Dodecyl Sulphate- Poly Acrylamide Gel Electrophoresis (SDS-PAGE): *S. aureus* enterotoxins were produced and concentrated using sac culture method of Donnelly *et al.* [13]. The protein concentration was estimated as described by Lowry *et al.* [14] and the SDS-PAGE procedure [15] was conducted in comparison to protein molecular weight marker (7-205 kDa) from Sigma.

Direct Detection of *S. Aureus* Enterotoxins in Food Samples Using Multiplex PCR: According to Nakayama *et al.* [16] each examined sample was added to an equal volume of 0.2 M sodium hydroxide and the mixture was incubated at 37°C for 20 min. The alkaline treated sample was neutralized with 10 ml 3 M sodium acetate (pH 5.4), extracted with 1.0 ml petroleum ether and then centrifuged at 13000 g for 10 min at 25°C. The aqueous phase was transferred to a fresh tube. Bacterial DNA was purified from the aqueous solution using a genomic DNA purification kit (Promega). Finally, DNA was eluted in 50 ml sterile distilled water and stored at 20°C. Then multiplex PCR technique was applied as previously mentioned.

RESULTS

Prevalence of *Staphylococcus* Species from the Examined Samples: As shown in Table 2, out of 250 examined food samples, 127 isolates were identified as *Staphylococcus* species (50.8 %), The highest rate of *Staphylococcus* species isolation was observed in raw milk samples (100%) followed by yoghurt samples, white soft cheese samples, meat and meat products (50% each) chicken products (46%) and pasteurized milk samples (16%). 32 *S. aureus* isolates were identified from the examined samples with an incidence of 12.8%. The highest isolation rate was observed in raw milk samples (56%) followed by yoghurt samples (22%), chicken products (6%), white soft cheese samples and pasteurized milk samples (4% each) then meat and meat products (2%).

Detection of Enterotoxigenic *S. aureus* Isolates: Using PCR, out of 32 *S. aureus* isolated from the examined food samples, 10 could produce enterotoxins as shown in Table 2 and Figure 1. Results revealed that out of five milk enterotoxigenic *S. aureus* isolates, 2 isolates were positive for *sea* gene, 1 isolate was positive for *sec* gene, 1 isolate was positive for *sed* gene and 1 isolate was positive for both types *seb* and *sed* genes. Out of four yoghurt enterotoxigenic *S. aureus* isolates, 1 isolate was positive for *seb* gene, 1 isolate was positive for *sed* gene, 1 isolate was positive for *see* and 1 isolate was positive for both *sea* and *sec* genes. Enterotoxigenic *S. aureus* isolated from Chicken product had both *seb* and *sed* genes. *S. aureus* isolated from cheese and meat products samples did not have enterotoxin genes.

SDS Profile Analysis of Enterotoxigenic *S. aureus* Isolates: Enterotoxins extracted from 7 enterotoxigenic *S. aureus* isolates (2 raw milk, 4 yoghurt and 1 chicken product sample) and *S. aureus* strain (ATCC 25923) were analyzed by SDS-PAGE as shown in Table 3 and Fig. 2. It is clear that all enterotoxigenic isolates had one or two bands at 26-29 kDa.

Direct Detection of *S. aureus* Enterotoxins in Food Samples Using Multiplex PCR: As shown in Fig. 3, both *sea* and s*ec* could be detected from yoghurt and raw milk samples, both *seb* and *see* could be detected from chicken product and raw milk samples and *sed* could be detected from yoghurt and raw milk samples.

Table 2: Prevalence of enterotoxigenic *S. aureus* isolated from the collected food samples evaluated by multiplex PCR.

	Staphylococcus species		*S. aureus*		Enterotoxigenic *S. aureus*		Genotypes						
Samples	No	%*	No	%*	No	%**	*sea*	*sea+sec*	*seb*	*seb+sed*	*sec*	*sed*	*see*
Raw milk n=25	25	100	14	56	5	35.7	2	0	0	1	1	1	0
Pasteurized milk n=25	4	16	1	4	0	0	0	0	0	0	0	0	0
Yoghurt n=50	25	50	11	22	4	36.4	0	1	1	0	0	1	1
White soft Cheese n=50	25	50	2	4	0	0	0	0	0	0	0	0	0
Meat and meat products n=50	25	50	1	2	0	0	0	0	0	0	0	0	0
Chicken products n=50	23	46	3	6	1	33.3	0	0	0	1	0	0	0
Total n=250	127	50.8	32	12.8	10	31.3	2	1	1	2	1	2	1

*Percentage was calculated to the number of the examined samples.

**Percentage was calculated to the number of *S. aureus* isolates.

Table 3: The relationship between the presence of 26-29 kDa and enterotoxins among the examined S. *aureus* isolates.

Lane no.	Origin of *S. aureus*	Mol. Wt. (kDa)	Enterotoxin typed by PCR
M	Marker	-	-
1	Yoghurt	26	*Sed*
2	Yoghurt	26	*See*
3	Yoghurt	26	*Seb*
4	Milk	28	*Sec*
Standard strain	Control positive (ATCC 25923)	26	*Seg*
5	Chicken	29	*seb + sed*
6	Milk	29,27	*seb + sed*
7	Yoghurt	28	*sea + sec*

Mol. Wt. = molecular weight kDa= kilo Dalton

Fig. 1: Agarose gel electrophoresis showing the results of multiplex PCR for detection of enterotoxin genes among the *S. aureus* isolates.
Lane M: 100 bp ladder DNA molecular weight marker (Axygen), Lane + ve: positive control for *seb, sed, sec, see* and *sea* genes, Lane-ve: negative control, a) Lane 1: positive *see S. aureus* isolated from yoghurt sample, Lane 2: positive *sed S. aureus* isolated from yoghurt sample, Lanes 3,7,8&11: no amplification, Lane 4: positive *sec* and *sea S. aureus* isolated from yoghurt sample, Lane 5: positive *seb S. aureus* isolated from yoghurt sample, Lane 6: positive *seb* and *sed S. aureus* isolated from chicken product, Lane 9: positive *sed S. aureus* isolated from raw milk sample and Lane 10: positive *seb* and *sed S. aureus* isolated from raw milk sample. b) Lanes 11 to19: no amplification. c) Lane 20: positive *sec S. aureus* isolated from raw milk sample, Lanes 21 to 24 and 27&28: no amplification, Lane 25: positive *sea S. aureus* isolated from raw milk sample and Lane 26: positive *sea S. aureus* isolated from raw milk sample. d) Lanes 29 to 32 no amplification.

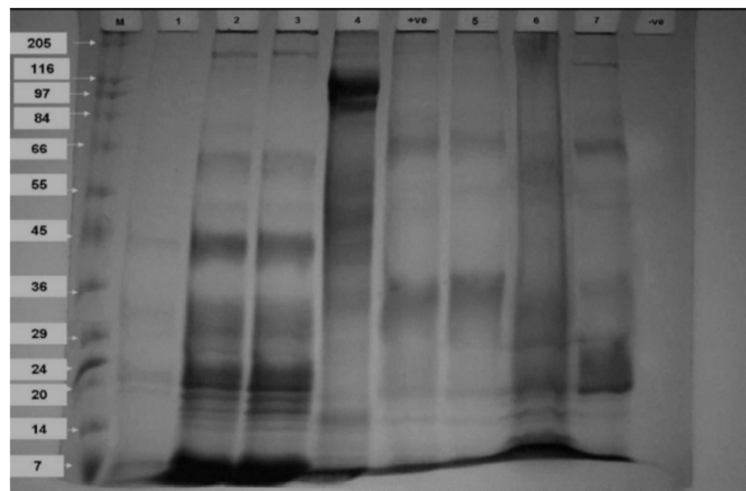

Fig. 2: SDS profile analysis of the 8 *S. aureus* isolates.
Lane M: protein molecular weight marker (7-205 kDa), Lane +ve: *S. aureus* strain (ATCC 25923), Lane-ve: negative control, Lanes 1, 2, 3and 7: *S. aureus* from yoghurt sample, Lanes 4 and 6: *S. aureus* from raw milk sample and Lane 5: *S. aureus* from chicken product sample.

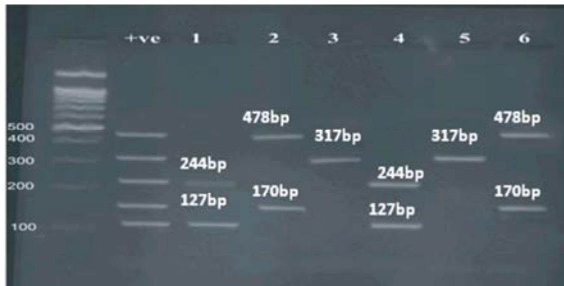

Fig. 3: Direct detection of *S. aureus* enterotoxins genes in food samples using multiplex PCR.
Lane M: DNA molecular weight marker (100bp ladder), Lane +ve: positive control for s*eb*, s*ed*, s*ec*, s*ee* and s*ea*, Lane 1: positive amplification of 244 bp for s*ec* and 127 bp for s*ea* from yoghurt sample, Lane 2: positive amplification of 478 bp for s*eb* and 170 bp for s*ee* from chicken product sample, Lane 3: positive amplification of 317 bp for s*ed* from yoghurt sample, Lane 4: positive amplification of 244 bp for s*ec* and 127 bp for s*ea* from raw milk sample, Lane 5: positive amplification of 317 bp for s*ed* from raw milk sample and Lane 6: positive amplification of 478 bp for s*eb* and 170 bp for s*ee* from raw milk sample.

DISCUSSION

S. aureus is an important food borne pathogen and a major cause of food poisoning outbreaks worldwide. The presence of *S. aureus* in ready to eat food which are eaten without cooking could be a bacterial risk for humans [17]. In the present study a total of 250 food samples collected from supermarkets and butchers in Giza and Cairo Governorates (25 raw milk samples, 25 pasteurized milk samples, 50 white soft cheese samples, 50 yoghurt samples, 50 meat and meat products and 50 chicken products) were investigated bacteriologically to detect the occurrence of enterotoxigenic *S. aureus* among the examined food samples. Routine diagnostic and identification procedure for detection of *Staphylococcus* isolates includes: isolation of colonies on mannitol salt agar and Baird-Parker agar for 24-48 hrs at 37°C, followed by coagulase assays for suspicious colonies and further confirmation by biochemical tests were carried out [18]. Coagulase positive *Staphylococcus* isolates are usually needed for additional tests to differentiate *S. aureus* other than coagulase positive staphylococci. Voges-Proskauer test was used to differentiate *S. aureus* from other coagulase positive species; *S. hyicus* and *S. intermedius* [8]. Table 2 illustrated that out of 250 examined food samples, 127 isolates were identified as *Staphylococcus* species (50.8 %), The highest rate of *Staphylococcus* species isolation was observed in raw milk samples (100%) followed by yoghurt samples, white soft cheese samples, meat and meat products (50% each) chicken products (46%) and pasteurized milk samples (16%). 32 *S. aureus* isolates were identified from the examined samples with an incidence of 12.8%. The highest isolation rate was observed in raw milk samples (56%) followed by yoghurt samples (22%), chicken products (6%), white soft cheese samples and pasteurized milk samples (4% each) then meat and meat products (2%). In China, raw meat, milk and dairy products, frozen products and cooked foods have been found as major food types contaminated by *S. aureus*, taking up 38%, 20%, 16% and 14%, respectively [18]. Milk is a good substrate for *S. aureus* growth and enterotoxin production. In addition, enterotoxins retain their biological activity even after pasteurization [19]. Imani Fooladi *et al.* [20] concluded that 32% of all dairy products were contaminated by *S. aureus*.

Staphylococcus species were detected from the examined white soft cheese samples and yoghurt samples with an incidence 50% as shown in Table 2. Staphylococci food poisoning resulting from contaminated milk and dairy products, especially cheeses produced from raw milk in unclean conditions, causes staphylococcal intoxication [21]. Differences between the results may be based on the differences in the cheese production techniques, storage conditions, type of cheese and whether the milk used was raw or pasteurized. It could be also related to the unclean conditions where the cheese is produced and the personnel involved in production. Among meat samples, it is clear that *Staphylococcus* species were detected from 50% of the examined meat and meat product samples and *S. aureus* could be identified with an incidence of 2%. Also *Staphylococcus* species were detected from 46% of the examined chicken products and *S. aureus* could be identified with an incidence of 6% (Table 2). The processed meat products are public health hazard due to the possible presence of food borne pathogenic bacteria which cause toxicities and outbreaks [22]. *S. aureus* causes alimentary toxicosis and produces different extracellular products [23]. Generally, five classical staphylococci enterotoxin (SE) SEA to SEE are recognized. It was shown that about 95% of staphylococcal food-poisoning outbreaks were caused by strains carrying the classical SE and the remaining 5% of outbreaks were associated with other identified [24].

There are several methods for detection of enterotoxigenic bacteria using serologic kits and PCR. Multiplex PCR may be considered more sensitive than immunological methods [25]. PCR is capable of detection of small amount of toxin in food samples. Using multiplex PCR, out of 32 *S. aureus* isolated from the examined food samples 10 could produce enterotoxins as shown in Table 2 and Fig. 1. Aydin *et al.* [2] analyzed 147 *S. aureus* isolated from 1070 food samples, for toxigenic capabilities, ninety-two strains (62.6%) isolated from meat, meat products, raw milk, dairy products, bakery products and ready-to-eat foods, were enterotoxigenic. Similar results were reported by Guven *et al.* [1] who isolated 138 *S. aureus* strains from 413 food samples collected in the central Anatolia region of Turkey and 83 (60.1%) of the strains synthesized one or two enterotoxins. In Portugal, Pereira *et al.* [26] observed that 101 out of 148 *S. aureus* isolates (68.2%) from various foods were positive for the presence of genes coding for one or more enterotoxins. In another study, Normanno *et al.* [27] found a nearly prevalence in Italy, in which 59.8% of the *S. aureus* strains isolated from milk, dairy and meat products produced enterotoxins.

In the current study enterotoxins were extracted from 7 enterotoxigenic *S. aureus* strains (2 raw milk, 4 yoghurt and chicken product samples) and analyzed by sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) analysis as shown in Table 3 and Figure 2. It is clear that all enterotoxigenic isolates had one or two bands at 26-29 kDa. SEs form heat-stable, water soluble, single-chain globular proteins with a molecular weight between 28,000 and 35,000 kDa [28]. Staphylococcal enterotoxins are low molecular weight proteins (26-29 kDa) [20]. The food industry aimed to develop a rapid method to test the safety of food. In the present work, six food samples (3 raw milk, 2 yoghurt and one chicken product samples) were analyzed using multiplex PCR as shown in Fig. 3. It is clear that both s*ea* and s*ec* could be detected from yoghurt and raw milk samples, both *seb* and *see* could be detected from chicken sausage and raw milk samples and s*ed* could be detected from yoghurt and raw milk samples. The direct multiplex PCR on food samples identified more SE-encoding genes than did on isolates indicating the sensitivity of PCR for direct detection of enterotoxigenic *S. aureus* in food samples.

It could be concluded that *S. aureus* producing enterotoxins present in the food samples, so most official regulations strictly require the absence of *S. aureus* form ready-to-eat foods. Multiplex PCR can highlight the presence of *S. aureus* representing the risk of toxicities.

REFERENCES

1. Guven, K., B.M. Mutlu, A.Gulbandilar and P. Cakir, 2010. Occurrence and characterization of *Staphylococcus aureus* isolated from meat and dairy products consumed in Turkey. J. Food Safety, 30: 196-212.
2. Aydin, A., K. Muratoglu, M. Sudagidan, K. Bostan, B. Okuklu and S. Harsa, 2011. Prevalence and antibiotic resistance of food borne *S. aureus* isolates in Turkey. Food borne Path. Dis., 8(1): 63-69.
3. Nagarajappa, S., M.S. Thakur and H.K. Manonmani, 2012. Detection of enterotoxigenic staphylococci by Loop-mediated isothermal amplification method. J. Food. Safety, 32: 59-65.
4. Bergdoll, M.S. and R.N. Robbins, 1973. Characterization of types Staphylococcal enterotoxins. J. Milk. Food. Technol., 36: 610-612.
5. Sergeev, N., D. Volokhov, V. Chizhikov and A. Rassoly, 2004. Simultaneous analysis of multiple staphylococcal enterotoxin genes by an oligonucleotid microarray assay. J. Clin. Microbiol., 42: 2134-2143.
6. Martin, M.C., J.M. Fueyo, M.A. Gonzales-Hevia and M.C. Mendoza, 2004. Genetic procedure for identification of enterotoxigenic strains of *Staphylococcus aureus* from three food poisoning outbreaks. Int. J. Food Microbiol., 94(3): 279-286.
7. Chiang, Y.C., W.W. Liao, C.M. Fan, W.Y. Pai, C.S. Chiou and H.Y. Tsen, 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 Microbiol., 121(1): 66-73.
8. Quinn, P.J., B.K. Markey, M.E. Carter, W.J. Donnelly, F.C. Leonard and D. Maguire, 2002. Veterinary Microbiology and Microbial Disease.1st Published, Oxford: Blackwell Science Ltd.
9. Sambrook, J., E.F. Fritsch and T. Maniates, 1989. Molecular Cloning: A laboratory Manual. 2nd Edition New York. Cold Spring Harbor Laboratory press, cold spring Harbor, N.Y. Publishing.

10. Pinto, B., E. Chenoll and R. Aznar, 2005. Identification and typing of food-borne *S. aureus* by PCR-based techniques. Syst. Appl. Microbiol., 28(4): 340-352.
11. Becker, K., R. Roth and G. Peters 1998. Rapid and specific detection of toxigenic *S. aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes and toxic shock syndrome toxin 1gene. J. Clin. Microbiol. 36(9): 2548-2553.
12. Johnson, W.M., S.D. Tyler, E.P. Ewan, F.E. Ashton, D.R. Pollard and K.R. Rozee, 1991. Detection of genes for enterotoxins, exfoliative toxins and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. J. Clin. Microbiol., 29(3): 426-430.
13. Donnelly, C.B., J.E. Leslie, L.A. Black and K.H. Lewis, 1967. Serological identification of enterotoxigenic Staphylococci from cheese. Appl. Microbiol., 15(6): 1382-1387.
14. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193(1): 265-275.
15. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
16. Nakayama, A., A. Okayama, M. Hashida, Y. Yamamoto, H. Takebe, T. Ohnaka, T. Tanaka and S. Imai, 2006. Development of a routine laboratory direct detection system of staphylococcal enterotoxin genes. J. Med. Microbiol., 55: 273-277
17. Odumeru, J.A., S.J. Mitchell, D.M. Alves, J.A. Lynch, A.J. Yee, S.L. Wang, S. Styliadis and J.M. Farber, 1997. Assessment of the microbiological quality of ready-to-use vegetables for health-care food services. J. Food Prot., 60(8): 954-960.
18. Xu, Z., L. Li, J. Chu, B.M. Peters, M.L. Harris, B. Li, L. Shi and M.E. Shirtliff, 2011. Development and application of loop-mediated isothermal amplification assays on rapid detection of various types of staphylococci strains. Food Res. Int., 47(2): 166-173.
19. Asao, T., Y. Kumeda, T. Kawai, T. Shibata, H. Oda, K. Haruki, H. Nakazawa and S. Kozaki, 2003. An extensive outbreak of staphylococcal food poisoning due to low fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. Epidemiol. Infect., 130(1): 33-40.
20. Imani Fooladi, A., H. Tavakoli and A. Naderi, 2010. Detection of enterotoxigenic *Staphylococcus aureus* isolates in domestic dairy products. Iran. J. Microbiol., 2(3): 137-142.
21. Can, H.Y. and T.H. Celik, 2012. Detection of enterotoxigenic and antimicrobial resistant *S. aureus* in Turkish cheeses. Food Control, 24(1-2): 100-103.
22. Rajic, A., L.A. Waddell, J.M. Sargeant, S. Read, J. Farber, M.J. Firth and A. Chambers, 2007. An overview of microbial food safety programs in beef, pork and poultry from farm to processing in Canada. J. Food Prot., 70(5): 1286-1294.
23. Yang, H., M.A. Xiaoyan, X. Zhang, Y. Wang and W. Zhang, 2011. Development and evaluation of a loop-mediated isothermal amplification assay for the rapid detection of *Staphylococcus aureus* in food. Eur. Food Res. Technol., 232: 769-776.
24. Wang, X., J. Meng, J. Zhang, T. Zhou, Y. Zhang, B. Yang, M. Xi and X. Xia, 2012. Characterization of *S. aureus* isolated from powdered infant formula milk and infant rice cereal in China. Int. J. Food Microbiol., 153(1-2): 142-147.
25. Holeckova, B., E. Holoda, M. Fotta, V. Kalýnacova, J. Gondol and J. Grolmus, 2002. Occurrence of enterotoxigenic *Staphylococcus aureus* in food. Ann. Agric. Environ. Med., 9(2): 179-182.
26. Pereira, V., C. Lopes, A. Castro, J. Silva, P. Gibbs and P. Teixeira, 2009. Characterization for enterotoxin production, virulence factors and antibiotic susceptibility of *S. aureus* isolates from various foods in Portugal. Food Microbiol., 26: 278-282.
27. Normanno, G., G. La Salandra, A. Dambrosio, N.C. Quaglia, M. Corrente, A. Parisi, G. Santagada, A. Firinu, E. Crisetti and G.V. Celano, 2007. Occurrence, characterization and antimicrobial resistance of enterotoxigenic *S. aureus* isolated from meat and dairy products. Int. J. Food Microbiol., 115: 290-296.
28. Dinges, M.M., P.M. Orwin and P.M. Schlievert, 2000. Exotoxins of *S. aureus*. Clin. Microbiol. Rev., 13(1): 16-34.