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การแพร่กระจายของยีนเอ็นเทโรทอกซินชนิดใหม่ (*seg, seh, sei, sej, and sel*) ในเชื้อสแตฟฟิโลคอคคัส ออเรียส ที่แยกได้จากอาหารสำเร็จรูปในภาคตะวันออกเฉียงเหนือของประเทศไทย

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Distribution of new Staphylococcal Enterotoxin Genes (*seg, seh, sei, sej, and sel*) in *Staphylococcus aureus* Isolated from Retail Ready-to-Eat Foods in the Northeast Thailand

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หลักการและวัตถุประสงค์: เชื้อ *Staphylococcus aureus* เป็นสาเหตุสำคัญที่ทำให้เกิดโรคอาหารเป็นพิษ อาหารสำเร็จรูปเป็นที่นิยมรับประทานกันในประเทศไทย การวิจัยนี้ทำการศึกษาการแพร่กระจายของยีนสร้างสารพิษเอ็นเทโรทอกซินชนิดใหม่ที่สำคัญ 5 ชนิดในเชื้อ *S. aureus* ที่แยกได้จากอาหารสำเร็จรูปที่ขายในจังหวัดขอนแก่น

วิธีการศึกษา: ใช้วิธีปฏิกิริยาลูกโซ่พอลิเมอเรส (PCR) ตรวจหายีนสร้างสารพิษเอ็นเทโรทอกซินชนิดใหม่ 5 ยีน (*seg, seh, sei, sej* และ *sel*) ในเชื้อ *S. aureus* 57 สายพันธุ์ที่แยกได้จากอาหารสำเร็จรูปที่สุ่มตรวจจำนวน 151 ตัวอย่าง ที่วางขายในอำเภอเมือง จังหวัดขอนแก่น โดยวิเคราะห์ข้อมูลร่วมกับยีนของสารพิษเอ็นเทโรทอกซินชนิดดั้งเดิม ที่ศึกษาก่อนหน้านี้แล้ว (*sea-sed* and *tsst-1*).

ผลการศึกษา: ตรวจพบยีนสร้างสารพิษเอ็นเทโรทอกซินชนิดใหม่ร้อยละ 29.8 (17 สายพันธุ์) โดยพบ ยีน *sea* ร่วมกับ *seg* จำนวนมากที่สุด (ร้อยละ 12.3) ตามด้วยยีน *seg+sei* (ร้อยละ 8.8) และยีน *sec+seg* (ร้อยละ 1.7) นอกจากนี้ยังพบยีน *seg* แบบเดี่ยวๆ จำนวน 4 สายพันธุ์ (ร้อยละ 7.0) แต่ไม่พบยีน *seh, sej* และ *sel*

สรุป: การตรวจพบยีนสร้างสารพิษเอ็นเทโรทอกซินชนิดใหม่ในเชื้อ *S. aureus* ที่แยกจากอาหารสำเร็จรูป ในอำเภอเมืองขอนแก่น พบว่ายีน *seg* และ *sei* พบได้จำนวนมาก และมักพบร่วมกับยีนสร้างสารพิษเอ็นเทโรทอกซินชนิดดั้งเดิม ชี้ให้

Background and Objective: *Staphylococcus aureus* is a common cause of food poisoning. Various ready-to-eat (RTE) foods have become increasingly popular in Thailand. The aim of this study was to investigate the distribution of enterotoxigenic *S. aureus* strains carring the newly important enterotoxin-encoding genes isolated from retail RTE foods in Khon Kaen municipality, Thailand.

Methods: In this study, polymerase chain reaction (PCR) primers specific for the detection of newly staphylococcal enterotoxin (*se*); encoding genes including *seg, seh, sei, sej* and *sel* were used for the assay of 57 *S. aureus* isolates from 151 RTE food samples randomly collected from food vendors and food shops in Khon Kaen municipality that have been previously investigated for the five classical enterotoxin genes (*sea-sed* and *tsst-1*).

Results: The result showed that the new enterotoxins could be found in 29.8% (17 of 57 isolates). The *sea* coexisted with *seg* was the most frequently found (12.3%), following by *seg+sei* (8.8%) and *sec+seg* (1.7%). Four isolates (7.0%) had single *seg* and none of *S. aureus* isolates had *seh, sej* and *sel.*

Conclusion: These findings indicated that some new enterotoxin genes such as *seg*, and *sei* were the most frequently found and frequently coexists with other classical enterotoxin genes. Therefore, these new

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เห็นว่าสารพิษเอ็นเทโรทอกซินชนิดใหม่ อาจมีส่วนทำให้เกิดโรคอาหารเป็นพิษในอาหารสำเร็จรูปได้

คำสำคัญ: เชื้อ *Staphylococcus aureus*, โรคอาหารเป็นพิษ, สารพิษเอ็นเทโรทอกซินชนิดใหม่

enterotoxins may play a role to cause food poisoning in RTE foods in Khon Kaen, Thailand.

Keywords: *Staphylococcus aureus*, food poisoning, newly staphylococcal enterotoxin

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Introduction

Staphylococcus aureus is an important pathogen associated with Staphylococcal food poisoning worldwide including Thailand[1,2]. According to the case outbreak in Thailand between January and December, 2014, the Bureau of Epidemiology, Ministry of public health reported a morbidity rate of food poisonings were 191.70 per 100,000 population and higher rate in North and Northeast Thailand[3].

S. aureus can produce more than 30 different extracellular enzymes and toxins[4]. Staphylococcal toxins can be categorized into many groups such as Staphylococcal enterotoxins (SEs), exfoliative toxins, toxic shock syndrome toxin-1, leucocidin and other toxins[5-7]. Generally, the classical staphylococcal enterotoxins i.e., SEA, SEB, SEC, SED, and SEE encoded by *sea*, *seb*, *sec*, *sed* and *see*, respectively, are recognized as the major cause of food poisoning[8-12]. Another enterotoxin, SEF, is biochemically identical to TSST-1[3,14]. The TSST-1 is also found to be associated with enterotoxins[15]. Some *S. aureus* can secrete SEs in contaminated food and they can apply to cooked food due to the thermostable character[16,17]. Moreover, the SEs and TSST-1 are known as bacterial super-antigens that can induce emetic activity, stimulating T cells and macrophages to release massive amounts of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α),interleukin (IL)-1, IL-6 and interferon-γ (IFN-γ) leading to capillary leakage, fever, hypotension and shock[18].

Recently, the new SEs are designated as SE-like (SEl) toxins because they lack the emetic properties[7,19]. Many new enterotoxins including SEG, SEH, SEI, SE/J, SE/K, SE/L, SE/M, SE/N, SE/O, SE/P, SE/Q, SER SES, SET, SE/U, SE/V and SE/W have been reported in other countries[20-26]. It has been known that approximately 95% of staphylococcal food poisoning outbreaks are caused by SEA to SEE[27] and the remaining 5% of outbreaks may be associated with other newly identified as SEs.

Various RTE foods have become increasingly popular in Thailand. Various microorganisms including *S. aureus* can be easily contaminated in RTE foods resulting in food poisoning[28,29]. Thai traditional foods especially green papaya salad ("somtum") and fermented pork mixed with rice ("nam krug") are popular local RTE foods. In addition, seafood and fresh fruit juice are also popular around the world[30].

In Thailand, our previous study has been reported the classical enterotoxin genes (*sea-sed* and *tsst*-1 and protein productions (SEA-SED and TSST-1) in RTE foods in Khon Kaen, Thailand[2], however, the new *SEs* genes have not been investigated.

Normally, many immunoassay kits such as the reverse passive latex agglutination and staphylococcal enterotoxin ELISA kits have been used for detection of classical enterotoxins (SEA-SED) and TSST-1[2]. Molecular methods such as DNA probes and polymerase chain reaction (PCR) assays, have been developed and used for the detection of these classical enterotoxins[31-34]. However, the detection of new SEs is not available because of lacking of commercial immunoassay kit in Thailand[24]. Therefore, the molecular assay PCR was used for detection these new enterotoxins in this study. As, the new enterotoxin genes, the *seg, seh, sei, sej* and *sel* genes were more frequently found in several types of foods [21,23-25,35-37]. Therefore, we choose to detect those enterotoxin genes in this study.

The aim of this study was to investigate the distribution of *seg, seh, sei, sej, and sel* combined with the classical staphylococcal enterotoxin genes (*sea-sed* and *tsst*-1 genes) that were isolated from RTE foods and patients in the Northeast Thailand. This study will be useful for understanding the prevalence of some staphylococcal new enterotoxins in food samples and clinical isolates.

Material and Methods

7.1 Bacterial strain

S. aureus strains used as reference strains or positive controls in this study were *S. aureus* ATCC 13565 (*sea*), ATCC 14458 (*seb*), ATCC 23235 (*sed*), ATCC 33586 (*tsst-1*), ATCC 19095 (*sec, seg, seh, sei* and *sel)* and *S. aureus* ATCC 23235 (*sej*). Fifty seven of *S. aureus* were isolated from 151 RTE food samples in Khon Kaen, Thailand.

7.2 Food sample collection and processing

The food samples collection and processing were performed as described previously[2]. In brief, 151 food samples were randomly collected from food vendors and food shops in Khon Kaen municipality, Thailand (20 food shops in Khon Kaen University and 60 food vendors and food shops outside Khon Kaen University). Food samples were grouped into three categories: 1) 50 samples of local foods (27 samples of green papaya salad, "somtum" and 23 samples of fermented pork mixed with rice, "nam krug"); 2) 50 samples of spicy seafood salad; and 3) 51 samples of fresh fruit juices and beverages. The food samples were aseptically collected and kept in sterile containers at 4 ºC prior to transfer to the laboratory.

A 25 g of each food sample (solid sample) was cut into small pieces and then, added to 225 ml of sterile Trypticase soy broth (TSB, Oxoid). The food sample mixture was incubated at 37 ºC for 18-24 hours, and then streaked on Baird-Parker plate containing egg yolk tellurite emulsion (Biomark, India) and incubated at 37 ºC for a further 48 hours[38]. Colonies typical of *S. aureus* (gray to jet-black surrounding opaque zone) were identified using biochemical tests[39].

7.3 DNA extraction

Genomic DNA of *S. aureus* was prepared by boiling method according to Perez-Roth et al[40]. In brief, *S. aureus* was grown overnight in brain heart infusion (BHI) with shaking at 37°C following with sedimentation at 13,000 g for 30 seconds. The bacterial pellet was re-suspended in sterile distilled water, boiled for 10 minutes, cooled on ice and sedimentation at 5,000 g for 1 minute. The supernatant was used as a template for PCR assay. A 5 µl of bacterial lysate was used directly as PCR template.

7.4 PCR assay

7.4.1 PCR Primer

The published primers were selected for detection of Staphylococcal new enterotoxin genes including *seh, sej* and *sel*. The new primers specific for *seg* and *sei* were designed and assessed in specificity. The primer sequences, amplification sizes and PCR condition are shown in Table 1. All primer pairs were checked for melting temperature, self-dimer, hetero dimer by using integrated DNA technology and test specificity of primer base on *in siligo* by BLAST software from the NCBI nucleotide public database (http:/ blast.ncbi.nlm.nih.gov/Blast.cgi) and then test specificity of primer using *S. aureus* reference strains as shown above with uniplex conventional PCR.

7.4.2 PCR mixture and PCR amplification

The oligonucleotide primers used in this study are listed in Table 1. For uniplex PCR, the amplification reaction was conducted in total volume of 30 µl contained 1X PCR buffer (contain 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl), 0.2 mM of deoxynucleotide triphosphate (dNTPs), concentration of primer including 0.5 µM for *seg, seh, sei* and *sej* or 0.6 µM for *sel* of each primer, 0.5 U of *Taq* DNA polymerase and 300 ng DNA template. After determined using the uniplex PCR with positive results, the duplex PCR of *sec* and *seg*, and *seg* and *sei* genes were also done.The amplification reaction was conducted in total volume of 30 µl containing 1X PCR buffer, 0.2 mM of dNTPs, concentration of primer including 0.5 µM for *sec* and *seg*, and *seg* and *sei* , 1 U of *Taq* DNA polymerase and 300 ng DNA template. PCR thermocyclings were performed using thermocycler (Bio-Rad C1000 thermal cycler).

7.4.3 Analysis of PCR products

The amplified products were analyzed by 1.5% agarose gel electrophoresis and stained with ethidium bromide before visualized by UV-transiluminator (Bio-Rad Gel™ Doc XR+ Imageer).

7.4.4 Statistic analysis

The distribution of newly staphylococcal enterotoxin genes was determined in percentage.

Table 1 The primer sequence and PCR condition.

Target gene	Primer sequence	PCR condition	Reference
sea (135 bp)	ACCGTTTCCAAAGGTACTGTA		Wongboot *et al.,*
	TGGTACACCAAACAAAACAGC		2013 [41]
seb (592 bp)	CCTAAACCAGATGAGTTGCAC	94°C ; 7 min	Wongboot *et al.,*
	CAGGCATCATGTCATACCAAA	35 cycles of	2013 [41]
sec (454 bp)	AGATGAAGTAGTTGATGTGTATGG	94 °C ; 30 sec/ 58 °C ; 30 sec/ 72 °C ;	Wongboot *et al.,*
	CTTCACACTTTTAGAATCAACCG	45 sec	2013 [41]
sed (263 bp)	GCTTGTACATATGGAGGTGTCA	72 °C ; 7 min	Wongboot *et al.,*
	GACCCATCAGAAGAATCAAACT		2013 [41]
tsst-1 (371 bp)	GGCAGCATCAGCCTTATAATTT		Wongboot *et al.,*
	GTGGATCCGTCATTCATTGTT		2013 [41]
seg (200 bp)	CTATACGAGTTTGATKGTTCT (*K= T or G)	94°C ; 5 min	This study
	CAGTGAGTATTAAGAAATACTTCC	35 cycles of	
sei (374 bp)	CAATTTCTTGAGCTGTKACTAGTT (*K= T or G)	94°C ; 1 min/ 58°C ; 1 min/ 68°C ; 1 min	This study
	AGGWGATATTGGTGTAGGTAACT (*W= T or A)	72°C ; 5 min	
seh (463 bp)	TCACATCATATGCGAAAGCAG	94°C ; 5 min	Cremonesi *et al.,*
	TCGGACAATATTTTTCTGATCTTT	35 cycles of	2005 [25]
		94°C ; 1 min/ 56°C ; 1 min/ 68°C ; 1 min	
		72°C ; 5 min	
sej (306 bp)	GGT TTT CAA TGT TCT GGT GGT	94°C , 5 min (35 cycle)	Cremonesi *et al.,*
	AAC CAA CGG TTC TTT TGA GG	35 cycles of	2005 [25]
sel (240 bp)	CAC CAG AAT CAC ACC GCT TA	94°C ; 1 min/ 53°C ; 1 min/ 72°C ; 1 min	Cremonesi *et al.,*
	CTG TTT GAT GCT TGC CAT TG	72°C ; 5 min	2005 [25]

Results

The enterotoxigenic types of *S. aureus* strains isolated from food samples obtained from Khon Kaen province in Thailand were investigated. The results are shown in Table 2 and Figure 1. Fifty seven *S. aureus* were isolated form 151 RTE foods. The finding of new enterotoxins showed that *sea* combined with *seg* was the most commonly found (12.3%), following with *seg* combined with *sei* (8.8%). The *sec* coexisted with *seg* was also found (1.7%). There were only four of these 57 isolates (7.0%) harbored *seg* and none of which harbored the *seh, sej* and *sel*. Local food showed the most harboring the new enterotoxin genes. We also determined those new enterotoxin genes in *S. aureus* strains isolated from diarrheal patients. Although, the number of isolates was small (4 isolates), 25% (1 isolate) harbored *seg* and *sei* together (data not shown).

Table 2 Information of staphylococcal enterotoxin genes detected by PCR assay.

Source		Number of sample	Number of *S.aureus* isolates	Number of toxin-positive and type of toxin (%)				
				seg	*sea+seg*	*sec+seg*	*seg+sei*	total
Food (n=151)	Local food	50	24	2 (8.3)	3 (12.5)	0	3 (12.5)	8 (33.3)
	Sea food	50	17	2 (11.8)	1 (5.9)	1 (5.9)	1 (5.9)	5 (29.4)
	Fruit juice and beverage	51	16	0	3 (18.7)	0	1 (6.3)	4 (25.0)
	Total	151	57	4 (7.0)	7 (12.3)	1 (1.7)	5 (8.8)	17 (29.8)

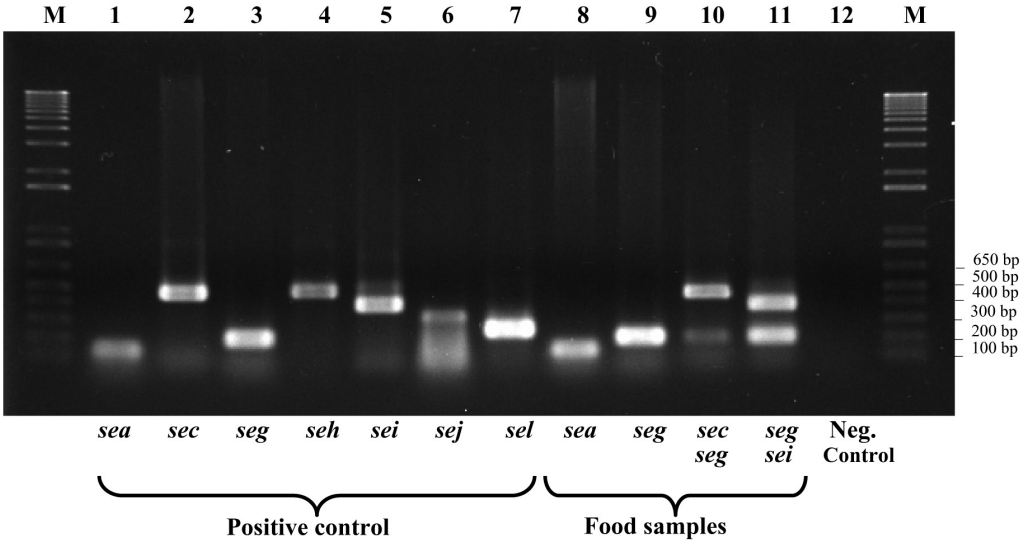

Figure 1. Gel electrophoresis for PCR products of Staphylococcal enterotoxin (*se*) genes. Lane M: 1 kb DNA ladder. Lane 1-7: *sea* (135 bp), *sec* (454 bp), *seg* (200 bp), *seh* (463 bp), *sei* (374 bp), *sej* (306 bp) and *sel* (240 bp) positive control. Lane 8-11: *sea, seg, sec+seg* and *seg+sei* of food samples. Lane 12: negative control.

Discussion

Previous study, the classical enterotoxin genes (*sea-sed* and *tsst*-1) were determined in RTE foods obtained from Khon Kaen province, Thailand[2]. The result showed that 60% was positive for presence of those classical enterotoxin genes and *sea* (46%) was the most common classical enterotoxigenic type to be found[2]. The precent study, we further determined some of new *S. aureus* enterotoxin genes (*seg, seh*, *sei, sej* and *sel*) in *S. aureus* strains isolated from the same RTE food samples in order to understand the emergence and distribution of new *se* in the RTE foods in Khon Kaen for indication of the risk of RTE foods in this province.

The local foods, "namkrug" and green papaya salad, gently heated foods may promote *S. aureus* contamination from unhygienic hand contact and/or raw materials[2,30]. Our previous study showed that the local foods were the highest of carring the classical enterotoxin genes which are similar with these new enterotoxin genes in the present study[2]. Our result indicated that the new enterotoxin genes were found in 29.8%. Although, most *S. aureus* strains harbored the classical enterotoxins, especially *sea*[2], the combination with other new enterotoxin genes was also found in RTE foods similar to other previous studies[22,23,26,37]. Other country studies showed that some of new *S. aureus* enterotoxin genes that the most recently described were *seg, seh, sei, sej* and *sel*[25,42]. Coexistence of *sea, seb, sec, sed* with other new enterotoxins -*seg, seh* and *sei* has been reported by other investigators[26,43,44]. These findings showed that

the variation of new Staphylococcal enterotoxin gene types was found in foods and can be explained with the epidemiology in each region and the type of foods that detected[21-24,35,36,45] such as Mashouf and colleagues reported that 35.7% of *seg* had more frequently found than classical *se(s)* in *S. aureus* isolated from milk, dairy product and raw meats[37], Omoe and colleagues reported that 38.9% of *seg* and *sei* was the most frequently found in *S. aureus* isolated from raw milk[26].

In this study, we also determined those new enterotoxins in *S. aureus* strains isolated from diarrheal patients. Although, the number of *S. aureus* isolated from diarrheal patients was small (4 isolates), 25% harbored *seg* and *sei* together (data not shown). Overall result of staphylococcal enterotoxin genes in *S. aureus* strains isolated from RTE foods and diarrheal patients in Khon Kaen, it can indicate that the major enterotoxins caused food poisoning were the classical enterotoxins[2], whereas the new enterotoxins may involve to cause food poisoning in Khon Kaen, Thailand. The result is in agreement with a previous report in Taiwan that showed that *S. aureus* strains isolated from fecal specimens of patients who were sick from food poisoning outbreaks in Taiwan were mostly positive for classical enterotoxins (67.8%), such as *sea* or *see* and some carried *seg*, *seh, sei* and *seg + sei*[23]. For the new enterotoxin, the *seg* was suggested to be detected in association with *sei*[46,47]. In this study, some strains were found to carry *seg* alone similar to McLauchlin *et al.*[46] reported. As, most *S. aureus* strains harbored the classical enterotoxins (60%) in previous study[2] and when combined with this report, over 90% of *S. aureus* strains isolated from RTE foods in Khon Kaen harbored the enterotoxin genes, indicated that RTE foods carried a risk for staphylococcal food poisoning.

Conclusion

This study indicates that some *S. aureus* strains isolated from RTE foods in Khon Kaen harbored the new enterotoxins alone (*seg*) and in combination together (*sei* and *seg*) or combination with the classical enterotoxins (*sea* and *sec*). Therefore, the new enterotoxins may involve causing food poisoning in RTE foods in Khon Kaen, Thailand. However, it should be aware that the quantitative assessment of these enterotoxin levels should be more elucidated for indicating food-poisoning.

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Reference

1. Jackranukul C, Panvisavas N, Chaturongakul S. Detection of *Staphylococcus aureus* Enterotoxin Type I using qPCR. Chiang Mai J Sci 2013; 40: 143-50.
2. Wongboot W, Chomvarin C, Namwat W. Phenotypic and genotypic detection of enterotoxins, toxic shock syndrome toxin-1 and of methicillin resistance in *Staphylococcus aureus* Isolated from retail ready-to-eat foods in Northeastern Thailand. Southeast Asian J Trop Med Public Health 2015; 46: 97-104.
3. Bureau of Epidemiology. Epidemiological surveillance and investigation report 506 of food poisoning. Nonthaburi: Bureau of Epidemiology, Department of Disease Control Ministry of Public Health; 2014 (in Thai).
4. Rogolsky M. Nonenteric toxins of *Staphylococcus aureus*. Microbiol Rev 1979; 43: 320.
5. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med 1998; 339: 520-32.
6. Malachowa N, DeLeo FR. Mobile genetic elements of *Staphylococcus aureus*. Cell Mol Life Sci 2010; 67: 3057-71.
7. Lina G, Bohach GA, Nair SP, Hiramatsu K, Jouvin-Marche E, Mariuzza R. Standard nomenclature for the superantigens expressed by Staphylococcus. J Infect Dis 2004; 189: 2334-6.
8. Bergdoll M, Sugiyama H, Dack G. Staphylococcal enterotoxin. I. Purification. Arch Biochem Biophys 1959; 85: 62-9.
9. Bergdoll MS, Borja CR, Robbins RN, Weiss KF. Identification of enterotoxin E. Infect Immun 1971; 4: 593-5.
10. Casman EP, Bennett RW, Dorsey AE, Issa JA. Identification of a fourth staphylococcal enterotoxin, enterotoxin D. J Bacteriol 1967; 94: 1875-82.
11. Casman EP. Further serological studies of staphylococcal enterotoxin. J Bacteriol 1960; 79: 849.

12. Williams RJ, Ward JM, Henderson B, Poole S, O'Hara BP, Wilson M, et al. Identification of a novel gene cluster encoding staphylococcal exotoxin-like proteins: characterization of the prototypic gene and its protein product, SET1. Infect Immun 2000; 68: 4407-15.
13. Pinchuk IV, Beswick EJ, Reyes VE. Staphylococcal enterotoxins. Toxins 2010; 2: 2177-97.
14. Fueyo J, Mendoza MC, Rodicio MR, Muniz J, Alvarez M, Martín MC. Cytotoxin and pyrogenic toxin superantigen gene profiles of *Staphylococcus aureus* associated with subclinical mastitis in dairy cows and relationships with macrorestriction genomic profiles. J Clin Microbiol 2005; 43: 1278-84.
15. Johler S, Tichaczek-Dischinger PS, Rau J, Sihto HM, Lehner A, Adam M, et al. Outbreak of Staphylococcal food poisoning due to SEA-producing *Staphylococcus aureus*. Foodborne Pathog Dis 2013; 10: 777-81.
16. Le Loir Y, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. Genetics and Molecular Research 2003; 2: 63-76.
17. Eley AR. Microbial Food Poisoning. 2nd ed ed. London,UK: Chapman & Hall; 1996.
18. Ryan KJ, Ray CG. Medical Microbiology. In. Fourth Edition ed. United States: McGraw-Hill; 2004.
19. Omoe K, Imanishi Ki, Hu D-L, Kato H, Takahashi-Omoe H, Nakane A, et al. Biological properties of staphylococcal enterotoxin-like toxin type R. Infect Immun 2004; 72: 3664-7.
20. Argudin MA, Mendoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins (Basel) 2010; 2: 1751-73.
21. Aydin A, Sudagidan M, Muratoglu K. Prevalence of staphylococcal enterotoxins, toxin genes and genetic-relatedness of foodborne *Staphylococcus aureus* strains isolated in the Marmara Region of Turkey. Int J Food Microbiol 2011; 148: 99-106.
22. Bania J, Dabrowska A, Bystron J, Korzekwa K, Chrzanowska J, Molenda J. Distribution of newly described enterotoxin-like genes in *Staphylococcus aureus* from food. Int J Food Microbiol 2006; 108: 36-41.
23. Chen TR, Chiou CS, Tsen HY. Use of novel PCR primers specific to the genes of staphylococcal enterotoxin G, H, I for the survey of *Staphylococcus aureus* strains isolated from food-poisoning cases and food samples in Taiwan. Int J Food Microbiol 2004; 92: 189-97.
24. Chiang YC, Liao WW, Fan CM, Pai WY, Chiou CS, Tsen HY. 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. Int J Food Microbiol 2008; 121: 66-73.
25. Cremonesi P, Luzzana M, Brasca M, Morandi S, Lodi R, Vimercati C, et al. Development of a multiplex PCR assay for the identification of *Staphylococcus aureus* enterotoxigenic strains isolated from milk and dairy products. Mol Cell Probes 2005; 19: 299-305.
26. Omoe K, Ishikawa M, Shimoda Y, Hu DL, Ueda S, Shinagawa K. Detection of *seg, seh*, and *sei* genes in *Staphylococcus aureus* isolates and determination of the enterotoxin productivities of *S-aureus* isolates harboring *seg, seh,* or *sei* genes. J Clin Microbiol 2002; 40: 857-62.
27. Bergdoll MS. Enterotoxins. Staphylococci and staphylococcal infections 1983; 2: 559-98.
28. Oh SK, Lee N, Cho YS, Shin D-B, Choi SY, Koo M. Occurrence of toxigenic *Staphylococcus aureus* in ready-to-eat food in Korea. Journal of Food Protection® 2007; 70: 1153-8.
29. Xing X, Li G, Zhang W, Wang X, Xia X, Yang B, et al. Prevalence, antimicrobial susceptibility, and enterotoxin gene detection of *Staphylococcus aureus* isolates in ready-to-eat foods in Shaanxi, People's Republic of China. Journal of Food Protection® 2014; 77: 331-4.
30. Jay JM. Staphylococcal gastroenteritis. In: Modern food microbiology: Springer; 2000: 441-59.
31. Becker K, Roth R, Peters G. Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. J Clin Microbiol 1998; 36: 2548-53.
32. Chen TR, Hsiao MH, Chiou CS, Tsen HS. Development and use of PCR primers for the investigation of C1, C2 and C3 enterotoxin types of *Staphylococcus aureus* strains isolated from food-borne outbreaks. Int J Food Microbiol 2001; 71: 63-70.
33. Johnson WM, Tyler SD, Ewan EP, Ashton FE, Pollard DR, Rozee KR. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. J Clin Microbiol 1991; 29: 426-30.
34. Tsen HY, Chen TR, Yu GK. Detection of B, C types of enterotoxigenic *Staphylococcus aureus* using polymerase chain reaction. J Chinese Argic Chem Soc 1994; 32: 322.
35. Alibayov B, Zdenkova K, Sykorova H, Demnerova K. Molecular analysis of *Staphylococcus aureus* pathogenicity islands (SaPI) and their superantigens combination of food samples. J Microbiol Methods 2014; 107: 197-204.
36. Bianchi DM, Gallina S, Bellio A, Chiesa F, Civera T, Decastelli L. Enterotoxin gene profiles of *Staphylococcus aureus* isolated from milk and dairy products in Italy. Lett Appl Microbiol 2014; 58: 190-6.

37. Mashouf RY, Hosseini SM, Mousavi SM, Arabestani MR. Prevalence of enterotoxin genes and antibacterial susceptibility pattern of *Staphylococcus aureus* strains Isolated from animal originated foods in West of Iran. Oman Med J 2015; 30: 283-90.
38. Bennett R, Lancette G. *Staphylococcus aureus*, Chapter 12, rev. Jan. 2001. FDA Bacteriological analytical manual, 8th ed., Rev. A. AOAC International, Gaithersburg, MD 2001.
39. Bannerman TL, Peacock S, Murray P, Baron E, Jorgensen J, Landry M, et al. *Staphylococcus, Micrococcus*, and other catalase-positive cocci. Manual of clinical microbiology: Volume 1 2006: 390-411.
40. Perez-Roth E, Claverie-Martın F, Villar J, Mendez-Alvarez S. Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. J Clin Microbiol 2001; 39: 4037-41.
41. Wongboot W, Chomvarin C, Engchanil C, Chaimanee P. Multiplex PCR for detection of superantigenic toxin genes in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* isolated from patients and carriers of a hospital in northeast Thailand. Southeast Asian J Trop Med Public Health 2013; 44: 660-71.
42. Ercolini D, Blaiotta G, Fusco V, Coppola S. PCR-based detection of enterotoxigenic *Staphylococcus aureus* in the early stages of raw milk cheese making. J Appl Microbiol 2004; 96: 1090-6.
43. Su YC, Wong AC. Optimal condition for the production of unidentified staphylococcal enterotoxins. Journal of Food Protection® 1993; 56: 313-6.
44. Rosec JP, Gigaud O. Staphylococcal enterotoxin genes of classical and new types detected by PCR in France. Int J Food Microbiol 2002; 77: 61-70.
45. Hennekinne JA, De Buyser ML, Dragacci S. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiol Rev 2012; 36: 815-36.
46. McLauchlin J, Narayanan G, Mithani V, O'neill G. The detection of enterotoxins and toxic shock syndrome toxin genes in *Staphylococcus aureus* by polymerase chain reaction. J Food Prot 2000; 63: 479-88.
47. Munson SH, Tremaine MT, Betley MJ, Welch RA. Identification and characterization of staphylococcal enterotoxin types G and I from *Staphylococcus aureus*. Infect Immun 1998; 66: 3337-48.