Research Article

Prevalence of classical Staphylococcal enterotoxin genes of *Staphylococcus aureus* isolated from ready-to-eat food in Ho Chi Minh City, Vietnam

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Abstract

Contamination of *Staphylococcus aureus* in ready-to-eat (RTE) food is a leading cause of foodborne illness in Vietnam. The aim of this study was to analyze the frequency of classical Staphylococcal enterotoxin genes (*sea, seb, sec, sed,* and *see* genes) in *S. aureus* strains isolated from ready-to-eat food in Ho Chi Minh, Vietnam (76 strains of *S. aureus* were isolated from 200 random samples of ready - to - eat (RTE) food, which include pate, pork roll, barbecue pork, roasted pork and roasted duck, 40 samples of each), all the samples were collected from different fast food services on street in district 5, 6, 7 and 8 in Ho Chi Minh City, Vietnam. All the trains of *S. aureus* were stored at -70°C for analyzing Staphylococcal enterotoxin genes). The obtained results in this study indicated that out of 76 *S. aureus* strains, there are six strains (7.9%) carried SE genes: *sea* gene (1.32%) was detected in one pate sample (Dist. 8), *sec* gene was detected (3.95%) in three samples, which are two pork roll samples (Dist. 7 and 8) and one roasted duck sample (Dist. 8), and finally *see* gene was detected in one pate sample (Dist. 8) and in one pork roll sample (Dist. 8). This could be a serious public health risk and highlight the need to implement good hygiene practices.

Keywords: *SE genes, S. aureus, ready-to-eat food, Ho Chi Minh City.*

1. INTRODUCTION

Staphylococcus aureus is a bacterium associated with many food poisoning outbreaks in recent years in the southern of Vietnam, *S. aureus is* also known as one of three bacteria that cause common food poisoning after *Salmonella* spp. and *Clostridium pefringens.* Food contaminated with *S. aureus* up to a level of ≥ 10^5 CFU/g or bacteria carrying enterotoxin producing genes is a potential hazard causing food poisoning. The purpose of this study is to analyze the level of contamination of *S. aureus* on processed meat samples used directly in Ho Chi Minh City for the following reasons: processed meat belongs to matrix categories, which are easy to create ideal growing conditions for *S. aureus*. Besides that, storage conditions for these foods are not suitable after processing, especially temperature is not being controlled during storage and trading process, as well as hygienic conditions of the environment, sellers and Food and packaging supplies are unsafety. Processed meats are the matrix containing many nutrients with added salt content, thus creating conditions for this bacterium to grow. On the basis of collecting the level of contaminated *S. aureus* ready-to-eat (RTE) meats used to assess the situation of the contaminated level of this bacterium and combining the analysis of the rate of these bacteria

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carrying the classical toxin genes *ent* A, *ent* B, *ent* C, *ent* D and *ent* E (*sea, seb, sec, sed* and *see* genes), these are genes coding for classic enterotoxins that often involve intestinal toxins and a potential hazard cause food poisoning. This study focused on investigating the carrying rate of *S. aureus* toxin-coding genes isolated from processed meat products used directly in Ho Chi Minh City in 2017. Data of this study used to provide information to managers about food safety and consumers about the risk of this type of bacteria.

2. MATERIALS AND METHODS

2.1. Research subjects

The aim of the study is to analyze the prevalence of classical staphylococcal enterotoxin genes (*sea, seb, sec, sed* and *see* genes) of 76 *Staphylococcus aureus* strains isolated from ready-to-eat food (RTE) in Ho Chi Minh city.

2.2. Materials

76 *S. aureus* strains isolated from 200 random samples of ready - to - eat (RTE) food including pate, pork roll, barbecue pork, roasted pork, and roasted duck (40 samples of each), which were collected from different fast-food services on street in district 5, 6, 7 and 8 at Ho Chi Minh City, Vietnam. These *S. aureus* strains were stored at -70°C for analyzing Staphylococcal genes [4].

The DNA templates for *sea, seb, sec, sed,* and *sec* genes of *S. aureus* strains were sponsored by Prof. Sakazakii, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Japan.

2.3. Research Methods

2.3.1. Detection of staphylococcal enterotoxins genes sea, seb, sec, sed and see Extraction of bacteria genomic DNA

All 76 strains of *S. aureus* were stored at -70°C and cultivated on TSA agar (VM856858844, Merck, Germany). From there single colony was inoculated in 5 mL BHI broth at 37°C overnight, and 1 mL culture was extracted follow Transfer bacteria culture to a 15 mL centrifuge tube, and centrifuge (Mikro 200R, Hettich, Germany) at 1,000 g/10 min to pellet bacteria. Remove and discard the supernatant and resuspend the pellet in 1.5 mL PBS. Then incubate the suspended bacteria in a oven at 100°C/15 min and centrifuged at 5,000 g/min. Determine the concentration and purity of DNA by the A260/A280 ratio (Quawell, UV-VIS Spectrophotometer Q5000, U.S.A). A 5 µL aliquot of the bacterial lysate was used directly as a PCR template [5].

2.3.2. PCR reaction assay

PCR was used to analyze the following genes: *sea, seb, sec, sed* and *see*. The reaction was performed in a total 25 µL volume, containing 0.1 mM of each primer (IDT), 12 µL of 2X Master mix (dNTP, $MgCl_2$, and Tag DNA polymerase, Promega), nuclease-free water (Promega), and 100 ng genomic DNA. Primer details are shown in Table 1, PCR implication was performed using Eppendorf thermocycler (Mastercycle Nexus GX2) with the following cycles: initial denaturation for 4 min at 94°C, and then 30 cycles at 94°C for 2 min (denaturation), 55°C for 2 min (annealing) and 72°C for 1 min (extension). The final extension was performed at 72°C for 5 min [3, 6]. The PCR amplified samples were analyzed by electrophoresis for 30 min at 100 V

through a 2% agarose gel (1st BASE) in 0.5 X Tris/Borate/EDTA (0.09 M Tris-HCl, 0.09 M boric acid, 2 mM EDTA, pH 8.3, Promega). A 100 bp ladder (TaKaRa, Japan) was used for reference. The gel was a stain in a solution of DNA Diamond™ nucleic acid dye (Promega, USA) for 30 min and visualized by Dark Reader transilluminator (Clare Chemical Research, USA).

Table 1. *Primer and temperature used for detection of Staphylococcal Enterotoxin genes [3]*

Gene	*Primer*	*Sequence*	*Base pairs*
Sea	SEA-F	TTG GAA ACG GTT AAA ACG AA	120
	SEA-R	GAA CCT TCC CAT CAA AAA CA	
Seb	SEB-F	TCG CAT CAA ACT GAC AAA CG	478
	SEB-R	GCA GGT ACT CTA TAA GTG CC	
Sec	SEC-F	GAC ATA AAA GCT AGG AAT TT	257
	SEC-R	AAA TCG GAT TAA CAT TAT CC	
Sed	SED-F	CTA GTT TGG TAA TAT CTC CT	317
	SED-R	TAA TGC TAT ATC TTA TAG GG	
See	SEE-F	TAG ATA AAG TTA AAA CAA GC	170
	SEE-R	TAA CTT ACC GTG GAC CCT TC	

3. RESULTS AND DISCUSSIONS

3.1. Result of detection of Staphylococcal enterotoxin genes (*sea, seb, sec, sed* and *see*) isolated from *S. aureus* strains

Among all 76 *S. aureus* strains, the results show that there were six strains (7.9%) that carried at least one SE gene. The one gene for *sea*, three genes for *sec*, and two genes for *see* were found in 1.32 %, 3.95 %, and 2.63% of all strains respectively. In which, *sea* gene (1.32%) was detected in one pate sample (Dist. 8), *sec* gene was detected (3.95%) in three samples, which are two pork roll samples (Dist. 7 and 8) and one roasted duck sample (Dist. 8), and finally *see* gene was detected in one pate sample (Dist. 8)and one pork roll sample (Dist. 8).

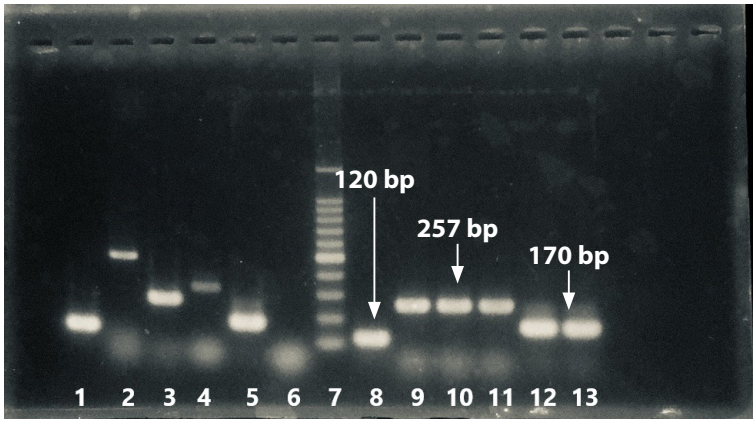

Figure 1. *Gel analysis of PCR amplified staphylococcal enterotoxin gene sequences; Lane 1: DNA positive for sea (120 bp); Lane 2: seb (478 bp); Lane 3: sec (257 bp); Lane 4: sed (317 bp), and lane 5: see (170 bp); Lane 6: blank sample (negative control); Lane 7: molecular ruler (100 bp); Lane 8: sea gene in pate (Dist. 8); Lane 9, 10, 11: sec genes (02 genes in pork roll (Dist. 7 and 8) and one gene in roasted duck (Dist. 8); Lane 12, 13: see genes (01 gene in pate and 01 gene in pork roll (Dist. 8).*

3.2. Discussions

According to the study of Bui Thi Mai Huong [1], the rate of contaminated *S. aureus* samples of meat products for RTE food in Hanoi was 22.2% and the rate of these strains carrying coding enterotoxin genes is 40% (detected by the RPLA enterotoxin typing), which is found to be mostly *sea*, *seb,* and *sec* genes. Considering the classic enterotoxin encoding genes found in our study is 7.9%, which is found to be mostly *sea, sec, and see* genes. But another study in Thai Lan [7], a total of 151 RTE food samples, collected from food vendors and food shops in Khon Kaen municipality, 38% (57/151) of food samples contaminates with *S. aureus*, with 60% (34/57) *S. aureus* isolates harboring super-antigenic toxin genes (*sea-sed*), in which *sea* 46%, *seb* 5%, *sec* 5%, *seb* + *sed* 3%. According to the research of Elisabetta Di Giannatale [2], 350 popular food samples were collected from retail outlets in the Abruzzi region of Italy. 14% of samples were contaminated with *S. aureus*. 18 out of 49 (16.3%) strains isolated from meat products were found to be positive for at least one coding gene for classical enterotoxin, as follows: 7 (14.0%) *sec* genes and 1 (2%) *sea* gene. Following a study in 2014, at Chengdu city of Sichuan province in China [8], in the 23 *S. aureus* strains isolated from cooked meat, 05 (21.7%) samples were found classical SE genes, in which *sea* gene in one out of 23 samples (4.35%), *seb* + *sec* in two out of 23 samples (8.7%) and *sea* + *sec* in two out of 23 samples (8.7%). *Sed* and *see* genes were not found in this study.

The prevalence of enterotoxin encoding *S. aureus* strains in processed meat in Ho Chi Minh city is different from the study in Hanoi, and some countries in ASEAN area also. This study shows that the common SE genes that *S. aureus* strains carried in RTE food in Ho Chi Minh, Vietnam are *sea, sec* and *see* genes. Meanwhile, *S. aureus* strains isolated in Hanoi, Vietnam contained *seb* and *sec*; in Thailand are *sea, seb*, *sec,* and *sed*; in China are *sea, seb,* and *sec*.

4. CONCLUSION

Our findings indicate that cooked meat products for ready to eat food revealed a risk for Staphylococcal food poisoning and that *sea, sec*, *sed* and *see* genes are the common SE genes detected in Ho Chi Minh, Vietnam. Appropriate hygienic measures should be taken by local public health organizations to reduce the risk posed by *S. aureus* in RTE foods.

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Tần suất mang gen độc tố ruột của các chủng *Staphylococcus aureus* phân lập được trên thực phẩm ăn ngay tại thành phố Hồ Chí Minh

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Tóm tắt

Tạp nhiễm *Staphylococcus aureus* trong thực phẩm ăn ngay, không qua chế biến (RTE) thường gây ra các ca ngộ độc thực phẩm tại Việt nam. Mục đích của nghiên cứu này nhằm phân tích tần suất mang gen sinh độc tố ruột (gen *sea, seb, sec, sed* and *see*) của các chủng *Staphylococcus aureus* đã phân lập trên các nền mẫu thực phẩm ăn ngay, không qua chế biến tại thành phố Hồ Chí Minh. 76 chủng *Staphylococcus aureus* được phân lập từ 200 mẫu thực phẩm lấy ngẫu nhiên từ các quầy bán thực phẩm trên đường phố, bao gồm patê, chả lụa, xá xíu, thịt heo quay, vịt quay được thu thập tại các Quận 5, 6, 7 và 8 tại thành phố Hồ Chí Minh. Các chủng Staphylococcus aureus được lưu giữ ở -70°C và được sử dụng để phân tích các gen sinh độc tố ruột. Kết quả nghiên cứu cho thấy, có 6/75 (7,9%) chủng *Staphylococcus aureus* mang gen sinh độc tố ruột, trong đó, 01 chủng mang gen sea (1,32%) trong patê (Quận 8), 03 chủng mang gen sec (3,95%) gồm 02 chủng trong chả lụa (Quận 7 và 8) và 01 chủng trong vịt quay (Quận 8) và 02 gen see (3,95%), trong đó 01 chủng trong patê (Quận 8) và 01 chủng trong chả lụa (Quận 8). Đây có thể là mối nguy cho sức khỏe công đồng và cần có những hướng dẫn thực hành vệ sinh tốt.

Từ khóa: *Gen sinh độc tố ruột, S.aureu, thực phẩm ăn ngay, thành phố Hồ Chí Minh.*