

Detection of Enterotoxin-Producing and Methicillin- and Vancomycin-Resistant *Staphylococcus aureus* in Ready-to-Eat Foods at Kasetsart University, Bangkheng Campus and Kaset Intersection Market

Praw Suppajariyawat^{1*}, Ong-ard Lawhavinit¹ and Patcharee Sunthornandh²

ABSTRACT

The objective of this study was to investigate the microbiological quality of ready-to-eat foods at Kasetsart University, Bangkheng campus and Kaset Intersection market. The enterotoxin producing, methicillin and vancomycin resistant *S.aureus* were found in 72 (33.33%) and 89 (41.20%) of food samples, respectively. During March to August 2008, 216 samples were collected from each of the two sampling sites. Of the 209 isolates of *Staphylococcus aureus*, 198 isolates were coagulase positive and 87 isolates were enterotoxin producing. The types of enterotoxin produced by these isolates were A, B, C, A&B, A&C and A&B&C&D, with the number of isolates being 15, 31, 10, 15, 15 and 1, respectively. The methicillin and vancomycin resistant strains of 87 isolates showed as 14.29% and 5.77% of methicillin and 11.43% and 6.90% of vancomycin, respectively from both sampling sites.

Key words: *Staphylococcus aureus*, enterotoxin, sensitivity test, methicillin resistant, vancomycin resistant

INTRODUCTION

Foodborne diseases affect people's health and well-being, as well as having an economic impact on individuals and nations. Approximately more than 120,000 cases of food poisoning were reported in Thailand each year (Bureau of Epidemiology, 2004). Bacteria are the causative agents of two thirds of foodborne outbreaks. Among the predominant bacteria involved in these diseases, *Staphylococcus aureus* is a leading cause of food poisoning, resulting from the consumption of contaminated foods. (Yves *et al.*, 2003). Furthermore, the increasing number of

antibiotic resistant strains of *Staphylococcus aureus* is a serious problem. (Jeljaszewicz and Hawiger, 1966). The emergence of antibiotic resistant microorganisms and their spread are concerning the medical community (Prakash *et al.*, 2007). This is particularly true in the case of *Staphylococcus aureus*. The enterotoxin producing strains and drug resistant strains, especially the methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Staphylococcus aureus* (VRSA) were occasionally in many kinds of foods, both raw and cooked (Phanukit *et al.*, 2005). Therefore, the objectives of this study were the detection of enterotoxin producing and

¹ Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.

² Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

* Corresponding author, e-mail: fvetonl@ku.ac.th

methicillin and vancomycin resistant *S.aureus* in ready-to-eat foods at Kasetsart University, Bangkok Campus and Kaset Intersection Market in order to examine the contamination of these strains in foods.

MATERIALS AND METHODS

Sample collection

The samples were grouped into three categories, that were 72 samples with a salty taste, 48 samples of bakery and 96 samples of dessert and fruit juice at six month intervals between March 2008 and August 2008. The sampling sites were four food shops at Kasetsart University, Bangkok campus and Kaset Intersection market, with 216 food samples collected at each sampling site. All samples were collected aseptically and placed in sterile containers, then transferred to the laboratory and stored at 4°C and analyzed immediately.

Enrichment and isolation

Initially, a 1:10 dilution was prepared with 25 g of each food sample in 225 ml trypticase soy broth (TSB, Pronadisa) at pH 7.3 with the addition of 10% sodium chloride (NaCl, Merck). The homogenized sample was incubated for 18-24 h at 35-37°C for enrichment, then spread onto Baird – Parker agar (BPA, Pronadisa) at pH 7.0. After incubation at 35-37°C for 18-24 h, the suspected colonies (black colony, convex and surrounded by clear zone of 2 to 5 mm in diameter and an opaque zone may appear inside the halo) were selected and transferred or restreaked on Baird-Parker agar. The pure culture of presumptive Staphylococci were subcultured and stored in nutrient agar (NA, Pronadisa) slant and maintained at -20°C, until use (Baron and Finegold, 1990; Bartelt, 2000).

Identification

All isolates were tested for gram stained, catalase test, coagulase enzyme, sugar

fermentation and tolerance to 10% NaCl. The isolated bacteria were identified according to the procedures presented by Baron and Finegold (1990) and Bartelt (2000).

Detection of enterotoxin production

S. aureus with Coagulase positive were grown on nutrient agar slant at 35-37°C for 18-24 h was transferred to 100 ml of trypticase soy broth. The flasks were incubated and shaken at 150 rpm 37°C for 18-24 h. The cultures were centrifuged at 10,000 rpm 4°C for 20 min. The supernatant was used to detect the Staphylococcal enterotoxins by Reverse Passive Latex Agglutination (RPLA) test kit (Oxoid). Staphylococcal enterotoxin reversed, passive latex agglutination (SET-RPLA) test kits included reference staphylococcal enterotoxins A,B,C and D solutions of latex particles sensitized with the corresponding anti-enterotoxins, a control latex solution, and diluent solution (0.05% phosphate-buffered saline, 0.5% bovine serum albumin and 0.05% sodium azide). The SET-RPLA test procedure involved the following steps. (i) A microtiter plate containing 12 rows of 96 U-shaped wells was labeled as necessary and six horizontal rows of eight wells each were reserved for testing toxin types A,B,C,D negative control and positive control. (ii) Supernatant (25 µl) was added to the first well of each of the five rows, but staphylococcal enterotoxin A was added to the sixth well. (iii) A solution of latex particles sensitized with anti-enterotoxins A, B, C, D, the latex control and anti-enterotoxin A were shaken and added to make up a volume of 25 µl to each well of rows 1 to 6, respectively. (iv) The microtiter plate was placed in a moist container, shaken on a rotating shaker at 150 rpm for 2 min and left in the container at room temperature overnight. (v) After the incubation period, the plate was observed against a black background. Tests were considered positive when agglutination was observed (Park and Szabo, 1986).

Resistant test for methicillin and vancomycin

The enterotoxin producing *S. aureus* were examined for methicillin and vancomycin resistance by Kirby-Bauer single disc diffusion testing (Bauer *et al.*, 1966). A 0.2 ml of 12 h peptone water of the test organism was used to inoculate a sterile nutrient agar plate, using a sterile glass spreader to cover the entire surface of the nutrient agar, and allowed to dry for about 15 to 30 mins. The antibiotic discs were placed on the agar using sterile forceps. Each disc was placed far from other discs to avoid intersection of the zones of growth inhibition. The plates with the antibiotic discs were then incubated at 37°C for 24 h to observe the zones of growth inhibition produced by the antibiotics. The zone of inhibition was measured and recorded according to the Clinical and Laboratory Standards Institute (CLSI).

RESULTS AND DISCUSSION

Staphylococcus aureus from ready-to-eat food samples collected from Kasetsart University, Bangkheng campus and Kaset Intersection market

The 432 food samples were examined for enterotoxin producing and methicillin and vancomycin resistant strains of *Staphylococcus aureus*. The results of the present study clearly indicated that the probability of finding *S. aureus* in food shops at Kaset Intersection Market was higher than that Kasetsart University, Bangkheng Campus (Table 1). In the 216 food samples from four food shops at the both sampling sites, 97 isolates of *Staphylococcus aureus* were found from

72 (33.33%) samples and also 112 isolates were found from 89 (41.20%) ready-to-eat food samples, respectively. The percentage of *Staphylococcus aureus* at six month intervals between March and August 2008 from ready-to-eat foods at both sampling sites is shown in Figure 1. In July 2008, a high level (52.78%, 66.67%) of contaminated foods was found, while there was a low level of contaminated foods in June, with 25.00 and 22.22% at Kasetsart University Bangkheng campus and Kaset Intersection market, respectively. The reason for the isolation of *Staphylococcus aureus* is that it is able to grow under a wide range of: temperature (7 to 48.5°C with an optimum of 30 to 37°C) and pH (4.2 to 9.3, with an optimum of 7 to 7.5), and it is tolerant to sodium chloride (up to 10% NaCl). These characteristics enable *Staphylococcus aureus* to grow in a wide variety of foods (Peterson *et al.*, 1964).

Detection of staphylococcal enterotoxins by the RPLA test

Table 2 shows that 198 of the 209 *S. aureus* isolates were coagulase positive and 87 isolates were enterotoxin producing. After detection of enterotoxin production by the RPLA kit test, the types of enterotoxin produced by these isolates were identified as A, B, C, A&B, A&C and A&B&C&D with the number of isolates being 15, 31, 10, 15, 15 and 1, respectively. The result concerning the detection of Staphylococcal enterotoxins by the RPLA who reported Isolation of enterotoxin producing *S. aureus* from Cafeteria Foods in Hospitals in Bangkok. The report revealed that Staphylococcal enterotoxin B (SEB)

Table 1 Number of *Staphylococcus aureus* positive ready-to-eat food samples at Kasetsart University Bangkheng campus and Kaset Intersection market.

Sampling site	Number of samples	Number of food samples isolated <i>S. aureus</i>
Bangkheng campus KU	216	72 (33.33%)
Kaset Intersection market	216	89 (41.20%)
Total	432	161 (37.27%)

was most frequently involved in food poisoning, but this result differed from Jay (2000), who reported that Staphylococcal enterotoxin A (SEA) was most frequently involved in food poisoning, followed by Staphylococcal enterotoxin D (SED). In the current study, SEB was found most frequently, indicating that there is a risk of staphylococcal food poisoning in foods. SEB is an important group of toxins implicated in several illnesses. SEB is a heat stable toxin and is not totally inactivated by the heat used in normal cooking and pasteurization (Minghui *et al.*, 2008)

Its potent enterotoxic and superantigenic properties have been reported as a causative agent in food poisoning and toxic shock, as well as autoimmune processes (Boris, 2008).

Methicillin and vancomycin resistance

The Kirby-Bauer method was used to detect methicillin and vancomycin resistance in the 87 enterotoxin producing *S. aureus* samples. The result revealed that isolates from ready-to-eat foods at both sampling sites showed a high percentage sensitivity to methicillin (85.71,

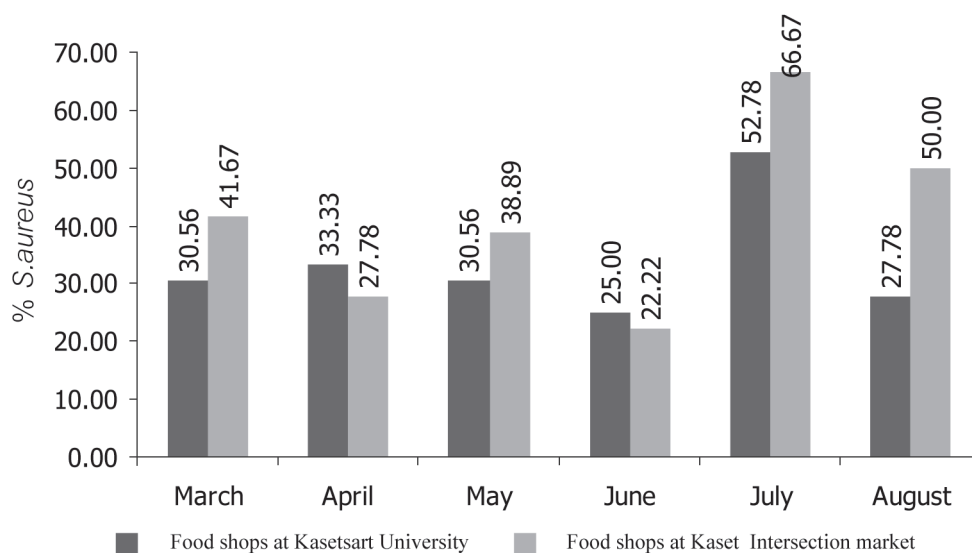


Figure 1 Percentage of *Staphylococcus aureus* at six month intervals between March and August 2008 from ready-to-eat foods at Kasetsart University, Bangkhen campus and Kaset Intersection market.

Table 2 Numbers of *Staphylococcus aureus* isolates with coagulase positive and enterotoxin producing strains.

Sampling site	Numbers of coagulase producing strains	Number of enterotoxin positive <i>S. aureus</i>	Type and number of enterotoxin producing strains				
			A	B	A&B	A&C	A&B&C&D
Bangkhen Campus KU	89	35(39.32%)	7	17	5	6	0
Kaset Intersection Market	109	52(47.70%)	8	14	10	9	1
Total	198	87(43.94%)	15	31	15	15	1

94.23%) and vancomycin (88.57, 96.15%), respectively (Table 3). In this study, the MRSA and VRSA strains were found in low level. Unfortunately, they were found in ready-to-eat food samples, indicating they are wide spread from hospitals to the wider environment. Therefore, it is difficult to predict future scenarios once *Staphylococcus aureus* acquires resistance to both established and newer antibiotics. It is essential to know the prevalence of drug resistance in any environment, because of the public health importance of a threat posed by MRSA and VRSA infection (Prakash *et al.*, 2007). Samples in the present study were handled carefully and there was every possibility that the spread of MRSA and VRSA could be enhanced by the carriers. Hence, it is suggested that measures to control MRSA and VRSA are urgently and immediately required. Considering the high numbers of indicator microorganisms and the discovery of pathogenic bacteria, food producers and consumers should cooperate in the management of foodborne pathogens to prevent and control outbreaks. Inappropriate consumption behavior (including eating raw or undercooked food and poor personal hygiene) and food contamination at any stage of food production, processing and delivery need to be assessed, monitored and improved. The

occurrence of drug resistant strains of *S. aureus* or other bacteria could be due to the misuse or overuse of antibiotics. The present study suggests that food contaminated MRSA and VRSA may constitute a health hazard to consumers.

CONCLUSION

A total of 97 and 112 isolates of *Staphylococcus aureus* were found from the 432 ready made food samples at Bangkhen campus, Kasetsart University and Kaset Intersection market. Of the 209 *Staphylococcus aureus* isolates, 198 were coagulase positive and 87 of 198 strains could produce enterotoxin. The types of enterotoxin produced were A, B, A&B, A&C and A&B&C&D. The results showed that MRSA and VRSA were found at low levels. However, the present study suggests that food contaminated by MRSA and VRSA may constitute a health hazard to the consumer.

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Table 3 Sensitivity test of the enterotoxin producing *Staphylococcus aureus* with methicillin and vancomycin.

Sampling site	Number of enterotoxin producing strains	Result (%)	Antibiotics	
			Methicillin	Vancomycin
Bangkhen Campus KU	35 (39.32%)	S	30(85.71%)	31(88.57%)
		I	0.0	0.0
		R	5(14.29%)	4(11.43%)
Kaset Intersection market	52(47.70%)	S	49(94.23%)	50(96.15%)
		I	0.0	0.0
		R	3(5.77%)	2(3.85%)
Total	87(43.94%)	S	79(90.80%)	81(93.10%)
		I	0.0	0.0
		R	8(9.20%)	6(6.90%)

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