Kasetsart J. (Nat. Sci.) 39 : 711 - 717 (2005)

Isolation of Drug Resistant and Enterotoxin Producing *Staphylococcus aureus* from Cafeterial Foods in Hospitals in Bangkok

Phanukit kunhachan[1], Chaivat Kitigul[1], Kantimanee Phanwichien[2] and Patcharee Sunthornandh[1]

ABSTRACT

The 525 food samples were examined for enterotoxin producing and drug resistance strains of *Staphylococcus aureus*. The 277 food samples from 12 government hospitals and 248 food samples from 12 private hospitals were examined. The 132 isolates of *Staphylococcus aureus* were found from 53 (19.13%) food samples of government hospitals and also the 100 isolates were found from 41 (16.53%) food samples of private hospitals. Two hundred and twenty six out of 232 isolates of *Staphylococcus aureus* were coagulase positive and 74 out of 226 coagulase positive strains could produce enterotoxin. The types of enterotoxin produced by these 74 isolates were A,B,C,A&B and A&C of 13,28,23,4 and 6 isolates, respectively.

The detection for drug resistance strains of 74 isolates that produced enterotoxin was doned by agar disc diffusion of Kirby-Bauer sensitivity test. The eight kinds of antibiotics and sulfonamides were selected base on the machanism of drugs, they were oxacillin, ciprofloxacin, rifampicin, vancomycin, gentamicin, tetracycline, chloramphenicol and co-trimoxazole. It was found that all isolates were sensitive to oxacillin,ciprofloxacin and rifampin (100%). Some were resisted to tetracycline (43.24%), chloramphenicol (17.57%) and gentamicin (4.05%), a few were intermediate to chloramphenicol, vancomycin and co-trimoxazole (trimethoprim/sulfamethoxazole). The multidrug-resistance strains were not found in this study.

Key words: staphylococcal enterotoxin, *Staphylococcus aureus*, drug resistance strains, cafeterial foods

INTRODUCTION

Staphylococcus aureus is a facultative anaerobe gram-positive coccus; it is non-motile, catalase and coagulase positive. Cells are spherical, single or paired arrangement, or form grape-like cluster (*Staphylo* means grape in Greek) (Murray *et al*., 1998). It could produce many kinds of enzyme and toxin which induced the host pathogenesis. This bacterium is a significant cause of nosocomial infections, as well as community-acquired disease. The spectrum of Staphylococcal infections ranges from pimples and furuncles to toxic shock syndrome and sepsis. On the other hand, some *Staphylococcus aureus* strains are able to produce Staphylococcal enterotoxins (SE_s) which have 12 types such as SEA,SEB, SEC_1, SEC_2, SEC_3, SED, SEE, SEG, SEH, SEI, SEJ (Balaban and Rasooly, 2000) and SEK (Orwin *et al*.,2001).

Staphylococcal enterotoxins (SE_s) are the causative agents of Staphylococcal food poisoning,

[1] Department of Microbiology, Facultry of science, Kasetsart University, Bangkok 10900, Thailand.
[2] Department of Zoology, Facultry of science, Kasetsart University, Bangkok 10900, Thailand.

Received date : 06/01/05 Accepted date : 04/05/05

the symptoms are intense abdominal cramps and diarrhea. The incubation period is about 1-6 hr. after consumption of food contaminated with enterotoxin or Staphylococci of 10^6 CFU per gram of food.

The emergence of drug resistant microorganisms in hospitals and the community is the problems of both treatment of patients and infection control which caused mortality of the patient in nosocomial infection. Organisms of particular concern include methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) (Chang *et al*., 1994; De Sousa *et al*., 1998; Gottlieb and Mitchell, 1998; Marchese *et al*., 2000).

In any case, the main sources of contamination are humans (handlers contaminated food via manual contact). These microbes are commonly transferred from patient to patient, on staff hands or is widely distributed in the environment such as air, dust, droplet nuclei or via the respiratory tract by coughing and sneezing and contamination occurs after heat treatment of the food (Lelievre et al., 1999).

The isolation of the enterotoxin producing strains and drug resistance strains, especially the MRSA and VRSA were occasionally found in many kinds of food , both raw and cooked, and were occasionally contaminated of these strains in food at cafeteria in hospitals both government hospitals and private hospitals in Bangkok.

The objectives of this study were the isolation of enterotoxin producing and drug resistance strains of *Staphylococcus aureus* from foods at cafeteria in hospitals in order to examined the contamination of these strains in foods.

MATERIALS AND MATHODS

Sampling The 277 food samples were collected from cafeteria of 12 government hospitals and 248 food samples from cafeteria of 12 private hospitals at three months intervals between 15 March 2002 and 15 June 2003. All food samples were transported to the laboratory within 2-3 hours after collection.

Enrichment and isolation Initially a 1:10 dilution was prepared with 25 g of food samples and liquid in 225 ml trypticase soy broth (TSB, Difco) at pH 7.3 with the addition of 10% sodium chloride. The homogenized sample was incubated for 18-24 h at 35 -37°C for enrichment. Suspension of organisms were inoculated onto petri dishes containing Baird – Parker agar (BPA, Difco) pH 7.0. After incubation at 35-37°C for 18-24 hr , selected suspected colonies (black colony, opaque zone and clear zone around the black colony) which were transferred and restreaked on Baird-Parker agar, incubated at 35-37°C for 18-24 hr. The pure culture of Staphylococci were subcultured and inoculated on nutrient agar slant (NA, Difco), incubated at 35-37°C for 24 hr. (Bartelt, 2000 ; Baron and Finegold, 1990).

Identification Preliminary, all isolates were examined by Gram stain, catalase test and coagulase test and identified according to the procedures presented by Bartelt (2000), Baron and Finegold (1990).

Procedure for enterotoxin production Preliminary, coagulase positive *Staphylococcus aureus* were inoculated on nutrient agar slant and incubated at 35-37°C for 18-24 h. After incubation, the coagulase positive *Staphylococcus aureus* were transferred to the flask containing 100 ml of trypticase soy broth (TSB, Difco). After incubation on incubator shaker at 150 rpm at 37°C for 18-24 h , the cultures were centrifuged at 10,000 × g at 4°C for 20 min. The supernatant was used for detection of Staphylococcal enterotoxins by RPLA (Reverse Passive Latex Agglutination kit test, Oxoid, Basingstoke, England) (Park and Szabo, 1986).

Detection of Staphylococcal enterotoxins by the RPLA test The Staphylococcal enterotoxin-reversed passive latex

agglutination (SET-RPLA) test kits were obtained from Oxoid Limited, Basingstoke, England. The kits included reference staphylococcal enterotoxins A,B,C and D, solutions of latex particle 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 was as follows: (i) A microtiter plate containing 12 rows of 96 U-shaped well was labelled as necessary and six horizontal rows of eight well 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 the sixth well was added with staphylococcal enterotoxin A. (iii) Solution of latex particles sensitized with anti-enterotoxins A,B,C,D, the latex control and anti-enterotoxin A were shaken and added in 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 the agglutination was observed (Park and Szabo, 1986).

Sensitivity test The 74 isolates that produced enterotoxin were examined for drug resistant by sensitivity test of Kirby-Bauer method which according to Tsen *et al* (1998). The eight kinds of antibiotics and sulfonamides were selected based on the mechanism of drugs, they were oxacillin, ciprofloxacin, rifampin, vancomycin, gentamycin, tetracycline, chloramphenicol and co-trimoxazole (Oxoid, Basingstoke, England).

RESULTS

The 525 food samples were examined for enterotoxin producing and drug resistance strains of *Staphylococcus aureus*. The 277 food samples from 12 government hospitals and 248 food samples from 12 private hospitals. *Staphylococcus aureus* were found from 53 (19.13%) food samples of government hospitals and 41 (16.53%) food samples of private hospitals. The result was shown in Table 1.

The 129 out of 132 isolates of *Staphylococcus aureus* which were isolated from 12 government hospitals (hospitalA-L) were coagulase positive (detected by coagulase rabbit plasma). After detection of enterotoxin production by RPLA kit test , 42 (32.56%) out of 129 coagulase positive strains could produce enterotoxin. The types of enterotoxin produced by these 42 isolates were A, B, C and A&C of 5, 13, 20 and 4 isolates, respectively.

The 97 isolates of 100 isolates of *Staphylococcus aureus* which were isolated from 12 private hospitals (hospital M-X) were coagulase positive. After detection of enterotoxin production by RPLA kit test , 32 (32.99%) out of 97 coagulase positive strains could produce enterotoxin. The types of enterotoxin produced by these 32 isolates were A, B, C, A&B and A&C of 8, 15, 3, 4 and 2 isolates, respectively. The result was shown in Table 2.

The detection for drug resistance strains of 74 isolates that produced enterotoxin was doned

Table 1 Number of *Staphylococcus aureus* positive food samples from government hospitals and private hospitals.

Type of hospital	Number of food samples	Number of food samples containing *S. aureus*
Government	277	53 (19.13%)
Private	248	41 (16.53%)
Total	525	94 (17.90%)

by Kirby-Bauer method. The eight kinds of antibiotics and sulfonamides were selected base on the mechanism of drugs, they were oxacillin, ciprofloxacin, rifampicin, vancomycin, gentamicin, tetracycline, chloramphenicol and co-trimoxazole. It was found that all isolates were sensitive to oxacillin, ciprofloxacin and rifampin (100%). Some resisted to tetracycline (43.24%), chloramphenicol (17.57%) and gentamicin (4.05%), a few were shown intermediate to chloramphenicol, vancomycin and co-trimoxazole (trimethoprim/sulfamethoxazole) (Table 3). An example of sensitivity test of *Staphylococcus aureus* strain K 8/3 shown in Fig 1.

DISCUSSION

Staphylococcus areus is normally found in the nostrils, and on the skin and hair of warm-blooded animals. Up to 30-50% of the human population are carriers. *Staphylococcus aur*eus is able to grow in a wide range of temperatures (7 to 48.5°C with an optimum of 30 to 37°C), pH (4.2 to 9.3, with and optimum of 7 to 7.5) and tolerant

Table 2 Number of the isolates of coagulase positive *Staphylococcus aureus* and the enterotoxin producing strains.

Type of hospita	Soure of samples	Number of isolates of coagulase positive *S. aureus*	Number of isolates of enterotoxin producing strains	Type and number of enterotoxin
Government	Hospital A	12	2 (16.67%)	2A
	Hospital B	5	- (-)	-
	Hospital C	11	5 (45.45%)	3B, 2C
	Hospital D	10	3 (30.00%)	1C, 2A&C
	Hospital E	15	- (-)	-
	Hospital F	16	4 (25.00%)	3B, 1C
	Hospital G	16	4 (25.00%)	4C
	Hospital H	9	2 (22.22%)	2C
	Hospital I	17	9 (52.94%)	2B, 5C, 2A&C
	Hospital J	3	- (-)	-
	Hospital K	8	8 (100.00%)	3A, 5B
	Hospital L	7	5 (71.43%)	5C
Total of government		129	42 (32.56%)	5A, 13B, 20C, 4A&C
	Hospital M	3	- (-)	-
	Hospital N	4	2 (50.00%)	2A&B
	Hospital O	5	2 (40.00%)	2A&C
	Hospital P	15	6 (40.00%)	6B
	Hospital Q	16	6 (37.50%)	4A, 2C
	Hospital R	113	(27.27%)	3B
	Hospital S	3	- (-)	-
	Hospital T	12	1 (8.33 %)	1B
	Hospital U	18	9 (50.00%)	2A, 5B, 2A&C
	Hospital V	4	- (-)	-
	Hospital W	-	- (-)	-
	Hospital X	6	3 (50.00%)	2A, 1C
Total of private		97	32 (32.99%)	8A, 15B, 3C, 4A&B, 2A&C
Total		226	74 (32.74%)	13A, 28B, 23C, 4A&B, 6A&C

Note : Number of positive samples are shown in the front of enterotoxin type

to sodium chloride (up to 10% NaCl). These characteristics enable *Staphylococcus aureus* to grow in a wide variety of foods (Peterson *et al*.,1964), especially enterotoxin producing and drug resistance strains are considered a risk for public health. It remains a major cause of food poisoning because it can contaminate in food products during preparation and processing. The results of this study, showed that the probability to find *S. aureus* from cafeterial foods of government hospitals (19.13%) was higher than that of private hospitals (16.53%) (Table. 1). The type of enterotoxin which was most frequently found in cafeterial foods from both government hospitals and private hospitals was SEB (28 isolates), second was SEC (23 isolates), third was SEA (13 isolates), fourth were SEA&SEB (4 isolates) and finally were SEA&SEC (6 isolates). But the detection of food samples did not find the isolates which produced SED, SEA&SED, SEB&SEC and three or more types (Table 2).

According to Casman and Bennett (1963), staphylococcal enterotoxin A (SEA) is a leading cause of food poisoning more than Staphylococcal enterotoxin B (SEB) because *S. aureus* could be produced SEA before SEB, SEA was produced in exponential phase but SEB was produced in early stationary phase and because of pH for producing SEA was more closely to food pH than pH for producing SEB. On the other hand, in this study, the enterotoxin B was found higher than enterotoxin A due to food samples were collected in summer which the temperature was optimum for growth of *S. aureus*, especially for the strains which produced enterotoxin B and in variety of foods more than other types of enterotoxin producing strains. Thus, from this results, it was found highest of enterotoxin B producing strains.

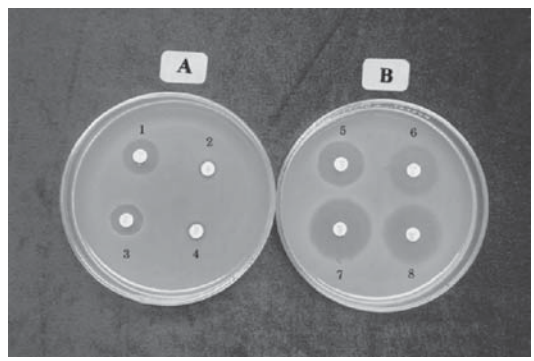

Figure 1 an example, sensitivity test of *Staphylococcus aureus* strain K 8/3.
A; 1 = oxacillin, 2 = chloramphenicol, 3 = vancomycin, 4 = tetracycline
B; 5 = gentamicin, 6 = ciprofloxacin, 7 = rifampin, 8 = trimoxazole/ sulfamethoxazole

Table 3 Sensitivity test of 74 isolates of enterotoxin producing *Staphylococcus aureus*.

Type of hospital	Result of sensitivity test	Antibiotics							
		OX	C	VA	TE	CN	CIP	RD	SXT
Government	% S	100	90.48	97.62	64.29	92.86	100	100	95.24
	% I	0.0	2.38	2.38	0.0	0.0	0.0	0.0	4.76
	% R	0.0	7.14	0.0	35.71	7.14	0.0	0.0	0.0
Private	% S	100	68.75	100	46.88	100	100	100	100
	% I	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	% R	0.0	31.25	0.0	53.12	0.0	0.0	0.0	0.0
Total	% S	100	81.08	98.65	56.76	95.95	100	100	97.30
	% I	0.0	1.35	1.35	0.0	0.0	0.0	0.0	2.70
	% R	0.0	17.57	0.0	43.24	4.05	0.0	0.0	0.0

Note : R, resistance; I, intermediate; S, sensitive; OX,oxacillin; C, chloramphenicol; VA, vancomycin; TE, tetracycline; CN, gentamicin; CIP, ciprofloxacin; RD, rifampin; SXT, trimoxazole/sulfamethoxazole

According to the results, the *Staphylococcus aureus* of enterotoxin producing and drug resistance strains were found in many kinds of food from both government and private hospitals. It should be the indicator to inform the consumers of unproper food hygiene and food sanitation of cookery and also the spread of *S. aureus* in the hospital environment. It should be transmitted from patients to the environment and then to the food or from the cooks themselves to the food. The occurrance of drug- resistance strains of *S. aureus* or other bacteria could be due to the misuse or overuse of antibiotics. In some cases it indicated that the use of same antibiotic to treat the patient continuously for a long time, induced the drug-resistance strains. Therefore, to prevent the resistance to the antibiotics of microbes doctors should be careful of using the antibiotics.

In this study, the drug-resistance strains were found in the low level and the multidrug-resisistance strains were not found. The MRSA and VRSA, the serious problem of nosocomial infection in many countries(Chang *et al*., 1994; De Sousa *et al*., 1998; Gottlieb and Mitchell, 1998; Marchese *et al*., 2000) and caused highly mortality rate, fortunately they were not found in all food samples, as shown in Table 3.

CONCLUSION

The 132 isolates of *Staphylococcus aureus* were found from food samples of government hospitals and the 100 isolates were found from private hospitals. The 226 *Staphylococcus aureus* isolates were coagulase positive and 74 positive strains could produce enterotoxin. The types of enterotoxin produced by these 74 isolates were A, B, C, A&B and A&C. 74 isolates were sensitive to oxacillin, ciprofloxacin and rifampin. Some were resistant to tetracycline, chloramphenicol and gentamicin, a few were intermediate to chloramphenicol, vancomycin and co-trimoxazole (trimethoprim/sulfamethoxazole). The MRSA and VRSA were not found.

LITERATURE CITED

Balaban, N. and A. Rasooly. 2000. Staphylococcal enterotoxins. **Int. J. Food Microbiol**. 61: 1-10.

Baron, E.J. and S.M. Finegold. 1990. **Diagnostic Microbiology**. 8th ed. The C.V. Mosby Company, United States of America. 861 p.

Bartelt, M.A. 2000. **Diagnostic Bacteriology : A Study guide**. F.A. Davis company, United States of America. 500 p.

Casman, E.P. and R.W. Bennett. 1963. Culture medium for the production of Staphylococcal enterotoxin A. **J. Bacteriol**. 85: 18-23.

Chang, S.C., W.C. Hsieh, and K.T. Luh. 1994. Fluoroquinolone resistance among methicillin- resistant Staphylococci after usage of fluoroquinolones other than ciprofloxacin in Taiwan. **Diagn. Microbiol. Infect. Dis**. 19 : 143-147.

De Sousa, A., I.S. Sanches, M.L. Ferro, M.J. Vaz, Z. Saraiva, T. Tendeiro, J. Serra and H. Lencastre. 1998. Intercontinental spread of a multidrug-resistant methicillin-resistant Staphyloccoccus aureus clone. **J. Clin. Microbiol**. 36 (9): 2590-2596.

Gottlieb, T. and D. Mitchell. 1998. The independent evolution of resistance to ciprofloxacin, rifampicin, and fusidic acid in methicillin-resistant *Staphylococcus aureus* in Australian teaching hospitals (1990-1995). **J. Ant. Chem**. 42: 67-73.

Lelievre, H., G. Lina, M.E. Jones, C. Olive, F. Forey, M.R. Delvallez, M.H.N. Chanoine, C.M. Bebear, V. Jarlier, A. Andremont, F. Vandenesch and J. Etienne. 1999. Emergence and spread in French Hospitals of methicillin-resistant *Staphylococcus aureus* with increasing susceptibility to gentamicin and antibiotics. **J. Clin. Microbiol**. 37(11): 3452-3457.

Marchese,A., G. Balistreri, E. Tonoli, E.A. Debbia and G.C. Schito. 2000. Heterogeneous vancomycin resistance in methicillin-resistant Staphylococcus aureus strains isolated in a large Italian Hospital. **J. Clin. Microbiol**. 38 (2): 866-869.

Murray, P. R., K.S. Rosenthal, G.S. Kobayashi and M.A. Pfaller. 1998. Medical Microbiology. 3rd ed., Mosby-Year Book, Inc., United States of America. 719 p.

Orwin, P.M., D.Y.M. Leung, H.L. Donahue, R.P. Novick and P.M. Schievert. 2001. Biochemicol and biological properties of Staphylococcal enterotoxin K. **Infect. Immun**. 69: 360-366.

Park,C.E. and R. Szabo. 1986. Evaluation of the reverse passive latex agglutination (RPLA) test kits for detection of Staphylococcal enterotoxin A,B,C, and D in foods. **Can. J.Microbiol**. 32: 723-727.

Peterson, A.C., J.J. Black and M.F. Gunderson. 1964. Staphylococci in competition III. Influence of pH and salt on staphylococcal growth in mixed populations. **Appl. Microbiol**. 12: 70-76.

Tsen, H.Y., G.K. Yu, K.C. Wang, S.J. Wang, M.Y. Chang and L.Y. Lin. 1998. Comparison of the enterotoxigenic types toxic shock syndrome toxin I (TSST-1) strains and antibiotic susceptibilites for enterotoxigenic *Staphylococcus aureus* strains isolated from food and clinical samples. **Food Microbiol**. 15: 33-41.