ENTEROPATHOGENIC BACTERIA AND ENTEROTOXIN-PRODUCING *STAPHYLOCOCCUS AUREUS* ISOLATED FROM READY-TO-EAT FOODS IN KHON KAEN, THAILAND

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Abstract. The objective of this study was to investigate the microbiological quality of ready-to-eat food in the Municipality of Khon Kaen, Thailand. Four categories of 186 food samples were collected: 1) high heat food; 2) low heat food; 3) no heat food; and, 4) on-site prepared fruit juices and beverages. Of the food samples, 145 (78%) failed to meet acceptable microbiological standards, including fruit juice and beverages (100%), no heat food (91.7%), low heat food (81.7%) and high heat food (57.9%). The most frequent bacterial indexes indicating unacceptability were the most probable number (MPN) of coliforms (78%), the bacterial colony count (58%), and the MPN of *E. coli* (46%). Pathogenic bacteria were found in 6.5% of food samples. *Salmonella, Vibrio cholerae* non O1 and *Aeromonas hydrophila* were found in 4.3, 1.6 and 0.5% of the total food samples, respectively. The serovars of *Salmonella* found in food were *S*. Derby, *S.* Give, *S.* Krefield, *S.* Paratyphi B, *S.* Verchow, *S.* Lexington and *S.* Senftenberg. *Staphylococcus aureus* concentrations of $>10^2$ CFU/g and $>10^5$ CFU/g were found in 10.8% and 1.1% of the food samples. Enterotoxin types AB and A of *S. aureus* were found in 2.7% of the food samples. These results indicate that more than half of the ready-to-eat foods tested in Khon Kaen municipality did not meet microbiological national standards and many kinds of enteropathogenic bacteria were found, suggesting food stalls may be a source of foodborne disease.

INTRODUCTION

Foodborne diseases affect people's health and well-being as well as have an economic impact on individuals and nations. Diarrheal disease has been a major public health problem causing high morbidity and mortality among children in Thailand for many years (Bureau of Epidemiology, 2004). Approximately one million cases of acute diarrhea, and more than 120,000 cases of food poisoning, are reported in Thailand each year (Bureau of Epidemiology, 2004). Food is considered the main source of microorganisms causing diarrheal diseases (Rabbani and Greenough, 1999; Jay, 2000; Fang *et al*, 2003).

Foodborne disease outbreaks from enteropathogenic bacteria, such as *Salmonella, Vibrio cholerae, V. parahaemolyticus* and *Staphylococcus aureus*, are common causes of foodborne infection throughout the world, including Thailand (Chomvarin *et al*, 1993; Mosupye and von Holy, 1999; Adams and Moss, 2000; Bangtrakulnonth *et al*, 2004; Meldrum *et al*, 2006). *Salmonella* caused diseases ranging from diarrhea to septicemia. Salmonellosis from contaminated food gen-

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erally causes diarrhea. *V. cholerae* and *V. parahaemolyticus* are the important *Vibrio* species causing watery-diarrhea and gastroenteritis (Jay, 2000; Murray, 2002; Montville and Matthews, 2005). *S. aureus* can also cause food poisoning even with very small amounts (100-200 ng) of its heat-stable enterotoxin (Evenson *et al*, 1988; Jay, 2000). *Aeromonas* spp are an emerging important pathogen causing diarrhea, and can be found in food and water (Kirov, 1993).

Microbiological criteria are used to distinguish between acceptable and unacceptable foods. They assess: 1) the safety of food for consumers, 2) adherence to good manufacturing practices, 3) the keeping quality (shelf life) of perishable foods, and 4) the suitability of a food or ingredient for a particular purpose (Montville and Matthews, 2005). Food is considered safe for consumers if there is : 1) an absence of pathogenic microorganisms and toxins, and 2) the types and numbers of microorganisms are within acceptable ranges (Montville and Matthews, 2005). Therefore, isolation of relevant bacterial pathogens and indicator organisms is used to evaluate microbiological safety and quality of food. Microbiological standards for ready-to-eat food in Thailand are determined by the guidelines of microbiological standards in food (1993) of the Department of Medical Sciences, Ministry of Public Health, Thailand. The following bacterial colony counts have been specified as maximum acceptable levels: bacterial colony count (BCC) (≤ 10^6 CFU/g), MPN of coliforms (≤500 CFU/g), MPN of *Escherichia coli* (≤ 3 CFU/g), and *S. aureus* (≤10^2 CFU/g), and the absence of pathogenic bacteria.

Ready-to-eat foods are processed (peeled, squeezed, cut up and/or cooked) and readily available for purchase and consumption. This type of food is popular among Thai consumers. However, ready-to-eat foods have been implicated in the transmission of foodborne disease (Chomvarin *et al*, 1993; Gillespie *et al*, 2000; Fang *et al*, 2003). Ready-to-eat foods at venders in Khon Kaen municipality were frequently left on the shelf too long, thus exposing them to bacterial contamination from the environment or food handing. *S. aureus* frequently causes food poisoning in Thailand, however, the types of staphylococcal enterotoxin in ready-to-eat foods have not been well studied (Chomvarin *et al*, 1993). Therefore, to investigate the types of staphylococcal enterotoxin and to establish the microbiological quality of ready-to-eat foods in Khon Kaen municipality, we investigated bacterial colony counts (BCC), two types of indicator organisms (*ie,* MPN coliform, MPN *Escherichia coli*), and selected foodborne pathogens, such as *Salmonella, Shigella, Vibrio,* and *S. aureus* (especially *S. aureus* producing enterotoxin) in ready-to-eat foods in Khon Kaen.

MATERIALS AND METHODS

Sample collection

One hundred eighty-six food samples were randomly collected from the food vendors and food shops in Khon Kaen municipality. The samples were grouped into four categories: 57 samples of no heat foods, 71 samples of low heat foods, 48 samples of high heat foods, and 10 samples of on-site prepared juices and beverages. All samples were collected aseptically and placed in sterile containers, stored at 4ºC, then transferred to the laboratory.

Bacterial colony count

Bacterial colony counts were performed according to the US Food and Drug Administration method (FDA, 1992) with modification. Briefly, 50 g of food sample was suspended in 450 ml phosphate-buffered saline (PBS), blended for 2 minutes, then diluted 10-fold to 1:10^5. Each dilution (0.1ml) was then spread-plated with a sterile glass rod onto plate count agar (Oxoid, Unipath Ltd, Basingstroke, Hamshire, United Kingdom). The plates were

incubated at 37ºC for 48 hours, then the colony count was reported as colony forming units per gram of food sample (CFU/g).

MPN of coliforms and *E. coli*

The MPN of coliforms was determined as described previously (Ohashi *et al*, 1978; Adams and Moss, 2000) Briefly, after homogenization as described in the bacterial count method, 10 ml, 1 ml and 0.1 ml of each diluted sample were inoculated into a series of five tubes of triple sets containing 10 ml of McConkey broth (Oxoid) at 37ºC for 48 hours. The MPN of coliforms was calculated from the number of the tube which showed bacterial growth and gas production. All positive tubes were sub-cultured onto Eosin methylene blue agar (EMB, Oxoid) and incubated at 35ºC for 18-24 hours. One to three typical colonies were picked up and identified as *E. coli* by biochemical test (Edwards and Ewing, 1986).

Detection and identification of *Salmonella*

The homogenate of 25 g of food sample in 225 ml of Trypticase soy broth (TSB, Oxoid) was incubated at 37ºC for 18-24 hours, followed by inoculating in selective enrichment of 1 ml in 9 ml of Tetrathionate broth (TT, Oxoid), Selenite-F broth (SF broth; Difco, Detroit, Michigan, USA). Then, the TT broth and SF broth were incubated at 37ºC for 18-24 hours and subcultured onto Salmonella-Shigella agar (SS agar, Oxoid) and Xylose lysine deoxycholate agar (XLD, Oxoid). The plates were then incubated at 37ºC for 18-24 hours.

After the homogenized food samples in TSB were incubated at 37ºC for 18-24 hours, the suspension was plated onto modified semisolid Rappaport medium (MSRV; Merck, Darmstadt, Germany) at 3 peripheral spots with 2 loops each, and then incubated at 42ºC for 18-24 hours. Positive colonies on MSRV were streaked onto XLD and Hektoen enteric agar (HE, Merck) and incubated at 37ºC for 18-24 hours. The suspected positive colonies were picked up and identified as *Salmonella* by conventional biochemical testing (Edwards and Ewing, 1986; Mahon and Manuselis, 2000).

The slide agglutination test with O-antigen (Biotechnical; Bangkok, Thailand) was used to group *Salmonella* isolates. The isolates were then submitted to the laboratory at the Department of Medical Science, Ministry of Public Health for further identification of serovars.

Detection and identification of *Vibrio* and *Aeromonas*

The 25 g of food samples were enriched in 225 ml alkaline peptone water (APW), pH 8.4 at 37ºC for 16-24 hours. A loop suspension was streaked onto Thiosulfate citrate bile salt sucrose agar (TCBS, Oxoid). The suspected colonies were identified by biochemical tests (Koneman, 1997). The *V. cholerae* colonies, were then determined for serogroup by slide agglutination with polyvalent *V. cholerae* O1 antiserum.

Detection of coagulase positive *Staphylococcus aureus*

Homogenized food sample in PBS (0.1 ml of 10^{-1} to 10^{-4} dilution) was spread onto plates of Baird Parker egg yolk agar (Oxoid) and incubated at 37ºC for 18-24 hours. The plates were then examined for typical *S. aureus* colonies (black, shiny, convex colonies with a narrow zone of opacity surrounded by a zone of clearing). A positive for *S. aureus* coagulase was determined by mannitol fermentation and coagulase production in human plasma.

Detection of *Staphylococcus aureus* enterotoxins

Production of enterotoxins A, B, C, D and TSST-1 was determined by a reverse passive latex agglutination kit (SET-RPLA, Oxoid) according to the manufacturer's instructions. A colony of coagulase-positive *S. aureus* was cultured in 1 ml of brain heart infusion broth and incubated at 37ºC for 18-24 hours. The culture was centrifuged and the supernatants

were tested for enterotoxin production using a passive latex agglutination kit.

RESULTS

Indicator microorganisms of ready-to-eat foods

The guidelines of microbiological standards in food by the Department of Medical Sciences, Ministry of Public Health, Thailand, were used to determine the quality of the different types of foods. Of 186 ready-to-eat food samples, 145 (78%) had unsatisfactory indicators and/or were positive for pathogenic microorganisms. Of the 186 food samples, 145 (78%), 108 (58.1%) and 86 (46.2%) had unsatisfactory counts of MPN of coliforms, BCC and MPN of *E. coli*, respectively. The types of food having unacceptable standards were: on-site produced fruit juice and beverages (100%), no heat foods (91.7%), low heat foods (81.7%) and high heat foods (57.9%) (Table 1).

Pathogenic bacteria

Of 186 food samples, *Salmonella* was found in 8 (4.3%) samples. Of 57 high heat foods, 71 low heat foods and 48 no heat foods, *Salmonella* was found in 1 (1.8%), 3 (4.2%) and 4 (8.3%) of these foods, respectively (Table 2). Different *Samonella* serovars were found in the different types of foods, particularly those with fermented pork sausage (Nam-mue) (Table 4). *Shigella* was not found in this study.

Table 1
Microbiological quality of 186 ready-to-eat foods according to the guidelines of microbiological standards in food of the Department of Medical Sciences, Ministry of Public Health, Thailand.

Types of food	Microbiological quality		Total samples
	Unacceptable	Acceptable	
High heat food	33 (57.9)	24 (42.1)	57
Low heat food	58 (81.7)	13 (18.3)	71
No heat food	44 (91.7)	4 (8.3)	48
Fruit juice and beverage	10 (100)	0 (0)	10
Total	145 (78)	41 (22)	186

Table 2
Types of pathogenic enteric bacteria and the enterotoxins produced by *S. aureus*, found in 186 food samples.

Type of food	No. of food samples	No. (%) of pathogenic bacteria and enterotoxins			
		Salmonella	*Vibrio cholerae* Non 01	*Aeromonas*	[Type of enterotoxin *of S. aureus*]
High heat food	57	1	2	-	-
Low heat food	71	3	1	1	2 [AB]
No heat food	48	4	-	-	2 [AB]
Fruit juice and beverage	10	-	-	-	1[A]
Total	186	8 (4.3)	3 (1.6)	1 (0.5)	5 (2.7)

Table 3
Range of cell counts in food samples positive for enterotoxin producing *S. aureus* and types of enterotoxins.

Types of food	Total food samples	Range of *S. aureus* (CFU/g)	No. of positive *S. aureus* (%)	Enterotoxin of *S. aureus* (%)	Types of enterotoxin	Name of foods
High heat food	57	10^2 - 10^5	2 (3.5)	0 (0)		
		>10^5	0 (0)	0 (0)		
Low heat food	71	10^2 - 10^5	4 (5.6)	1 (1.4)	AB	Vermicelli (Kanom Jean)
		>10^5	2 (2.8)	1 (1.4)	AB	Fermented pork mixed rice (Nam Krug)
No heat food	48	10^2 - 10^5	6 (12.5)	1 (2.1)	AB	Fermented fish (Nam Pla)
		>10^5	3 (6.3)	1 (2.1)	AB	Mushroom sour salad (Yum Hed)
Fruit juice and beverage	10	10^2 - 10^5	3 (30.0)	1 (10.0)	A	Ice coffee (O-Lieng)
		>10^5	0 (0)	0 (0)		
Total	186		20 (10.8)	5 (2.7)		

Vibrio cholerae non-O1 was found in 3 (1.6%) of 186 food samples. Two (3.5%) samples were isolated from high heat foods and 1 (1.4%) sample from low heat food. *Aeromonas hydrophila* was found in 1 (0.5%) of the food samples, it was low heat food (Tables 2, 4)

Staphylococcus aureus at unacceptable levels (>100 CFU/g) was found in 20 (10.8%) food samples (Table 3). Only 5 isolates (2.7%) produced enterotoxins. Four isolates produced enterotoxin AB (Staphylococcal producing enterotoxin A and B, SE AB) and one isolate produced only enterotoxin A (SE A) (Tables 2, 3). *S. aureus* counts in the samples showed a wide range of variation from not found to more than 10^5 CFU/g (Table 4).

DISCUSSION

This study shows that the majority (78%) of ready-to-eat foods sampled at vendors in Khon Kaen municipality were of unacceptable microbiological quality. Unsatisfactory results were indicated by high bacterial indicators (*ie*, MPN of coliforms, BCC and MPN of *Escherichia coli*) and bacterial pathogens (including *V. cholerae, Salmonella* and *S. aureus* producing enterotoxin). The types of food having an unacceptable standard included: on-site produced fruit juice and beverages (100%), no heat foods (91.7%), low heat foods (81.7%) and high heat foods (57.9%).

A high BCC alone does not make food unsafe but it does suggest non-hygienic handling, poor storage, inadequate general hygiene during processing and/or poor quality raw materials (Gillespie *et al*, 2000). Moreover, high MPN of coliforms and MPN of *E. coli* indicate the possibility of a microbial hazard and fecal contamination (Ayulo *et al*, 1994; Suwansonthichai and Rengpipat, 2003). High heat foods with large numbers of MPN of coliforms and MPN of *E. coli*, indicate the possibility of inadequate cooking (quick cooked) and/or post process-contamination.

Table 4
Numbers and types of pathogenic bacteria found in various types of foods.

Types of food	Total No. of samples	No. of pathogens (%)	Pathogenic bacteria		Name of foods
			Types	No. of isolates	
High heat food	57	3 (5.3)	*V. cholerae* nonO1	2	Quick fried bean cake with pork (Pud Toa Hu Mue Sub) Quick fried vegetables (Pud Puck Roum)
			S. Derby	1	Quick fried small noodle (Pud Mee)
Low heat food	71	5 (7.0)	*V. cholerae* nonO1	1	Squid sour salad (Yum Pramouk)
			S. Give	1	Fermented pork mixed rice (Nam Krug)
			S. Krefield	1	Fermented pork mixed rice
			S. Paratyphi B biovar Java	1	Tripe chicken grill (Krungnai gai yang)
			Aeromonas hydrophila	1	Pork sour salad (Larb Mue)
No heat food	48	4 (8.3)	*S*. Verchow	1	Fermented pork sausage (Nam Mue)
			S. Lexington	1	Fermented beef sausage (Nam Nour)
			S. Senftenberg	1	Fermented pork sausage
			S. Lexington	1	Fermented pork sausage
Fruit juice and beverage	10	0 (0)		0	
Total	186	12 (6.5)		12 (6.5)	

S. aureus at concentrations of $>10^2$ and $>10^5$ CFU/g was found in 10.8 and 1.1% of samples, respectively. However, *S. aureus* enterotoxins found in 2.7% of food samples, which is less than previous reports (Chomvarin *et al*, 1993). Some researchers found that 100-200 ng of enterotoxin produced by $>10^5$ CFU/g of *S. aureus* can cause gastroenteritis in healthy adults (Evenson *et al*, 1988; Ray, 2004). Our findings indicate a risk of staphylococcal food poisoning because some enterotoxins were found. Ten enterotoxins have been identified: A, B, C1, C2, C3, D, E, G, H, and I. Previous studies revealed that SE A was most frequently involved in food poisoning following by SE D (Jay, 2000). Some coagulase-negative *Staphylococcus* are also able to produce enterotoxins (Bergdoll, 1995). The symptoms of staphylococcal enterotoxins occur within 2-4 hours, with a range of 30 minutes to 8 hours, with vomiting and diarrhea (Ray, 2004). Our results show the foods with the

highest *S. aureus* contamination (*ie,* >10^5 CFU/g) were no heat and low heat foods and the enterotoxins were types A and AB. This finding indicates the major factors for *S. aureus* contamination may come from unhygienic handling and/or raw materials, probably from animal origin (Jay, 2000).

By the way of confirmation, *Salmonella* was found in 8 (4.3%) ready-to-eat food samples. Different serovars were found, mostly related to animal origin, especially pork. Our results agree with previous reports that *Salmonella* is transmitted to humans mainly via contaminated foods, especially of animal origin (Boonmar *et al*, 1998; Bangtrakulnonth *et al*, 2004). The difference in *Salmonella* serovars indicate the contamination occurred from sources of raw meat, as in previous reports (Boonmar *et al*, 1998; Bangtrakulnonth *et al*, 2004; Angkititrakul *et al*, 2005).

V. cholerae non O1 was found in 3 (1.6%) food samples. Two *V. cholerae* non O1 isolates came from high heat foods (quick-fried bean cake with pork) and one low heat food (squid sour salad). Our findings suggest the possibility of contamination of raw materials and inadequate cooking. Seafood may have been contaminated at the source, whereas the quick-fried bean cake with pork may have had post-processing contamination and/or inadequate cooking.

Regarding the high numbers of indicator microorganisms and the finding of pathogenic bacteria, organizations responsible for food safety, comprised of government agencies, 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. Our epidemiological data provide valuable baseline information suited for use by public health organizations and consumers.

We conclude that more than half of the ready-to-eat foods sampled in Khon Kaen municipality did not meet public health standards, suggesting food stalls may be a source of foodborne disease. Public health organizations should be concerned since microorganisms causing foodborne illness and food spoilage can be isolated from raw materials and finished food products; thus, reduction of contamination is an achievable policy objective.

ACKNOWLEDGEMENTS

This research was supported by Khon Kaen University, Thailand. We thank the staff (especially Mrs Pernpit Hanon and Ms Arunya Kongtavon) of the Department of Microbiology, Faculty of Medicine, for help doing some laboratory work, and Mr Bryan Roderick Hamman for assistance with the English-language.

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