Food Control 21 (2010) 221–226

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Prevalence of foodborne pathogens in open markets and supermarkets in Thailand

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ARTICLE INFO

Article history:
Received 22 January 2009
Received in revised form 8 May 2009
Accepted 19 May 2009

Keywords:
Listeria monocytogenes
Salmonella
Retail market contamination
Foodborne pathogens distribution
Thailand

ABSTRACT

This study was conducted in Thailand (Bangkok and Pathum Thani provinces), from June 2006 to July 2007, in order to assess the prevalence of *Listeria monocytogenes, Escherichia coli* O157, *Salmonella*, *Shigella* and *Vibrio parahaemolyticus* in foods. Retail raw meats and seafood, including chicken ($n = 109$), pork ($n = 80$), beef ($n = 108$), shrimp ($n = 43$) and oysters ($n = 48$), from open markets and supermarkets were analyzed. *Salmonella* was found in 22 of 61 (36%) open market samples (48% of chicken, none of pork and beef, and 53% of shrimp) and in 12 of 75 (16%) samples from supermarkets (57%, 12%, 24%, 0% respectively). However, a small number of *L. monocytogenes* were isolated, where 6 of 217 (3%) were samples from open markets (6% of chicken and 3% of pork) and 17 of 171 (10%) were from supermarkets (3% of beef, 4% of chicken, and 32% of pork). In both markets, *L. monocytogenes* was not detected from shrimps, neither from oysters. *E. coli* O157, *Shigella* and *tdh*-positive *V. parahaemolyticus* were not isolated in this collection. Several *Salmonella* and *L. monocytogenes* isolates were multidrug-resistant. Both markets would need better assessment, since multidrug-resistant strains have been isolated and they may lead to therapeutic failure.

1. Introduction

Thailand in the past years has been shown as an outstanding exporter of chicken meat and shrimp. Export products fulfill the international hygiene requirement standards, such as HACCP and GMP. Chicken and shrimp market chains show that exporter farms productions are not exclusive for export and part of their products are consumed in the domestic market as well (Sriwichailamphan, 2007). In the other hand, non-export producers with varied hygiene standard and production levels also supply the local retailers. The most consumed meat in the country is pork, followed by chicken and beef (FAO, 2007). There are basically two kinds of markets in Thailand: supermarkets and open markets. The supermarkets, which are indoor markets, often display prepackaged products under refrigeration. In contrast, the open markets usually display unwrapped products at ambient temperatures, exposing to potential contaminations.

Diarrheal diseases have been considered the major public health problem in Thailand. There are approximately more than 120,000 cases of reported food poisoning cases every year (FAO, 2004). Since foods are important vehicle of pathogens, identifying pathogens in outbreaks is critical in order to maintain awareness of ongoing problems (Olsen, MacKinon, Goulding, Bean, & Slutsker, 2000). *Salmonella, Listeria monocytogenes, Shigella, Vibrio parahaemolyticus* and *Escherichia coli* O157 are common bacterial agents involved with foodborne disease (CDC, 2007). The presence of these pathogens in raw food stuffs may result in further cross contamination and it may lead to public health consequences.

The objective of this study was to determine the prevalence and the antibiotic resistance of *Salmonella, E. coli* O157, *L. monocytogenes, V. parahaemolyticus* and *Shigella* in retail raw meats and seafood obtained in open markets and supermarkets from Bangkok and Pathum Thani provinces, Thailand, in order to provide better picture of the burdens due to pathogens commonly transmitted by food.

2. Materials and methods

2.1. Sample collection

Overall 388 samples including 108 beef, 109 chicken, 80 pork, 43 shrimp, and 48 oysters were analyzed in this study. Among

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doi:10.1016/j.foodcont.2009.05.011

which, 136 were analyzed for *Salmonella*, 388 for *L. monocytogenes*, 186 for *E. coli* O157, 91 for *Shigella* and 48 for *V. parahaemolyticus*. A total of 36 samplings were done in open markets and supermarkets located in Bangkok (two open markets and four supermarkets) and Pathum Thani (three open markets and one supermarket) during the period of June 2006 to July 2007. The sampling markets were chosen according to the convenience of distance from the laboratory. Sample number collected per market varied from 10 to 20 samples. The open markets are composed of booths where the meat vendors are specialized solely in one meat type, displaying the products in plastic trays with no covers, at ambient temperature, exposing them to potential contaminations such as insects and rodents. The seafood vendors in open markets often have ice directly on their products. Open market meat samples were cut, weighed, placed in a plastic bag and tied by the meat vendor, while seafood samples were taken by the vendors' bare hands. Supermarket samples were already pre-weighed and prepackaged in plastic wrap and displayed under refrigeration. Sampling criteria was limited to 200 g of one sample per vendor in open markets, and to one package per meat cut or seafood in supermarkets. Meat samples were limited to breast, thigh and wing for chicken, and steak and ground meat for cow and pork. No organ meat was sampled in this study. Shrimp were limited to entire samples, and oysters were limited to shelled samples. All samples were within the recommended date for consume, and were transported on ice and processed in the laboratory within 3 h after purchase. Sample is considered here as the each item purchased from the market, while isolate is the each strain of pathogen isolated as single colony from selective agars, and was characterized as distinct either in serotype or in antibiotic resistance.

2.2. Bacterial isolation

Modifications of methods described in the FDA CFSAN – Bacteriological Analytical Manual Online (FDA, 2001) were used to isolate *Salmonella* spp., *E. coli* O157, *L. monocytogenes*, *V. parahaemolyticus*, and *Shigella* spp. The isolation protocols for all pathogens proceeded with 25 g of each sample aseptically placed in a filtered stomaching bag (TGK-Tokyo Glass Kikai, Tokyo, Japan) and 225 ml of appropriate pre-enrichment broth added. The sample was homogenized in the stomacher (AES Laboratoire, France) for 1 min, sealed, and incubated at 37 °C for 24 h. Except for *L. monocytogenes*, which was incubated at room temperature for 48 h, incubation steps for all protocols were performed at 37 °C. Enrichment and selection processes are described below.

2.3. Salmonella

For *Salmonella* isolation, 225 ml of Buffered Peptone Water (OXOID, Basingstoke, Hampshire, England) was added to the sample. A 0.5 ml portion of overnight enrichment broth was added to 10 ml of Rappaport-Vassiliadis (MERCK, Darmstadt, Germany). After 24 h of incubation, the broth culture was streaked on DHL (Deoxycholate Hydrogen sulfide Lactose) agar, (Eiken Kizai, Tokyo, Japan) and CHROMagar Salmonella (CHROMagar, Paris, France) agar plates and incubated for 24 h. Suspected colonies were submitted to TSI (Triple Sugar Iron) (OXOID) and MIL (Motility Indol Lysin) (BD, Sparks, MD., USA) biochemical tests, and serotypes were determined by using Denka-Seiken (Tokyo, Japan) antisera following Kauffman-White serotyping scheme (Popoff, 2001).

2.4. E. coli O157

Samples were incubated for 24 h in mTSB (Modified Tryptic Soy Broth) (MERCK) to isolate *E. coli* O157. One milliliter of overnight broth was subcultured in 9 ml of mTSB. Following the incubation, one drop of immunomagnetic beads O157 (Denka-Seiken) was added to 1 ml of culture, and incubated according to the manufacturer's instructions. After washing step, it was suspended in 100 μl of phosphate buffered saline, from which each 50 μl was streaked on CHROMagar O157 TAM (CHROMagar) and on CT-SMAC (OXOID). Suspected colonies were submitted to biochemical TSI and MIL tests, and O157 antisera (Denka-Seiken) was used for serotype confirmation.

2.5. L. monocytogenes

The isolation of *L. monocytogenes* strains was detected by adding Half Fraser broth to samples and incubating for 48 h at room temperature. A 0.1 ml portion of broth was transferred to 10 ml Fraser Broth. A loopful of overnight culture was streaked on PALCAM (OXOID) and CHROMagar Listeria (CHROMagar) agars and incubated for 48 h. Presumptive colonies were submitted to sugar fermentation test (Rhamnose, Mannitol, Dextrose (Cica Kanto, Tokyo, Japan)) and Rhamnose positive strains were submitted to seroagglutination test (Denka-Seiken).

2.6. Shigella

Shigella Broth (OXOID) with novobiocin (Cica Kanto) and 1.5 g/l Tween 80 (Cica Kanto) was added to samples to detect *Shigella* and incubated for 24 h. A loopful of broth was streaked on Salmonella Shigella (OXOID) and on DHL agar, and incubated for 24 h. Presumptive colonies were submitted to biochemical TSI and MIL tests.

2.7. V. parahaemolyticus

Samples were homogenized in Phosphate Buffered Saline (PBS) with 3% NaCl. Subsequently, 10 ml of mixture was added to 90 ml of Alkaline Peptone Water (OXOID) and incubated for 24 h. A loopful of broth was streaked on TCBS (Thiosulfate Citrate Bile Salts Sucrose) agar (MERCK) and CHROMagar Vibrio (CHROMagar) plates. Following incubation, suspected colonies were submitted to tests of MIL, TSI and Nutrient Broth (BD) with 0%, 3%, 8%, and 10% of NaCl. Isolates with turbidity in 3% and 8% of NaCl were submitted to PCR analysis.

2.8. PCR assays

All suspected single colonies were subcultured on Tryptic Soy Agar (TSA) (BD) and analyzed by PCR. Targets, primer sequences, and PCR product sizes are shown in Table 1. The templates were prepared by boiling half to a loopful of colonies in 100 μl of distilled water for 10 min. PCR reagents were purchased from TaKaRa Bio (Japan). Each PCR reaction was processed independently, and the 25 μl reaction mixture contained the following: 1× reaction buffer (10 mM Tris pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP, dTTP, 10 pmol of each primer, 1 U of Taq polymerase, and 10 μl of bacterial template. PCR were ran in Takara PCR Thermal Cycler Dice with the following conditions: initial denaturation at 94 °C for 4 min; 30 cycles of amplification (denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 30 s), and final extension at 72 °C for 10 min. PCR products were stained with ethidium bromide and visualized under UV light after gel electrophoresis on 1% agarose.

2.9. Serotyping: *Salmonella*

Salmonella antisera were purchased from Denka-Seiken. Three to five times the amount of a match head of *Salmonella* grown overnight at 37 °C on TSA were emulsified with 0.5 ml of 0.85% saline to

Table 1
Targets and primers used in PCR assays for identification of virulence.

Bacteria	Target gene	DNA sequence (5′–3′)	Product size (bp)
Shigella	*ipaH*	TGTATCACAGATATGGCATGA TCCGGAGATTGTTCCATGTG	242
Salmonella	*stn*	CTTTGGTCATAAAATAAGGCG TGCCCAAAGCAGAGAGCTTC	260
L. monocytogenes	*hly*	CGCGGATGAATTCGATAC GTCATACCCGGGAAATCAATG	316
V. parahaemolyticus	*tdh*	GGTACTAAATGGCTGACATC CCACTACCACTCTCATATGC	251
E. coli O157	*rfbE*	CTACAGGTGAAGGTGGAATGG ATTCCTCTCTTTCCTCTGCGG	327
E. coli O157	*fliC*	TACCATCGCAAAAGCAACTCC GTCGGCAACGTTAGTGATACC	247
E. coli O157	*stx*	GAGCGAAATAACCCATATGAG TCCGGAGATTCTTCCATGTG	518
E. coli O157	*stx*	GAACGAAATAATTTATATGT TTTGATTGTTACAGTCAT	905

prepare the somatic antigen suspensions. Five microliters of suspension was rocked on slide glass for 1 min with one drop of respective somatic factor. The flagellar antigen suspensions were prepared by adding 6 ml of 1% formalin-treated saline to 6 ml of overnight culture in TSB (Tryptic Soy Broth), which was sit for 1 h prior to use. Five hundred microliters of suspension were incubated in glass tubes at 50 °C for 1 h with three drops of respective flagellar factors. Phase induction was performed using Craigie tubes in TSB with 0.3% agar.

2.10. Serotyping: L. monocytogenes

L. monocytogenes antisera were purchased from Denka-Seiken Overnight culture grown in BHI agar (Brain Heart Infusion) at 30 °C was diluted to 10 mg in wet weight per ml with 0.2% saline, autoclaved at 121 °C for 30 min and concentrated to prepare the somatic antigen suspension. One loopfull of suspension were rocked on slide glass for 1 min with one drop of respective somatic factor. To prepare the flagellar antigen suspensions, the culture was grown on BHI agar at 30 °C, where it was subjected to 5–6 passages through filter paper stripes (Whatman, GE Healthcare, USA) bridging cut agars. After the passages on cut agars, 4 ml overnight culture was prepared and 4 ml of 1% formalin-treated saline was added, which was sat for 1 h prior to use. Five hundred microliters of flagellar antigen suspension were incubated in glass tubes at 50 °C for 1 h with three drops of respective flagellar factors.

2.11. Antimicrobial susceptibility determination

Antimicrobial resistance of *Salmonella* and *L. monocytogenes* was determined by using disk diffusion test (Sensi-Disc, BD), following manufacturer's instructions and interpreted in accordance to the Clinical and Laboratory Standards Institute – CLSI (CLSI, 2006) guidelines. The antibiotics tested for *Salmonella* with their respective concentrations per disk in μg were: gentamycin 10 sulfisoxazole 25, chloramphenicol 30, ciprofloxacin 5, kanamycin 30, nalidixic acid 30, tetracycline 30, ampicillin 10 and streptomycin 10. Except for penicillin, ampicillin and trimethoprim-sulfamethoxazole, there are no susceptibility zone-diameter breakpoints for *Listeria* provided by the CLSI, therefore, we adopted *Staphylococcus* spp. criteria, as employed by other investigators (Troxler, von Graevenitz, Funke, Wiedemann, & Stock, 2000; Hansen, Gerner-Smidt, & Bruun, 2005). The antibiotics tested for *L. monocytogenes* with their respective concentrations per disk in μg were: amikacin 30, ampicillin 10, bacitracin 10, chloramphenicol 30, ciprofloxacin 5, clindamycin 2, erythromycin 14, gentamicin 10, imipenem 10, kanamycin 30, levofloxacin 5, minocycline 30, netilmicin 30, nitrofurantoin 300, penicillin 10, rifampicin 5, streptomycin 10, teicoplanin 30, tetracycline 30, tobramycin 10, trimethoprim- sulfamethoxazole 23.75/1.25 and vancomycin 30.

2.12. Statistical analysis

Chi-squared (χ^2) or two-sided Fisher's exact tests were used to analyze the data sorted by meat type, pathogen and market type. A probability value of less than 5% was considered to be significant.

3. Results and discussion

This study describes the prevalence of foodborne pathogens in open markets and supermarkets in Thailand. *Salmonella* and *L. moncytogenes* were the only pathogens detected in this collection. Analyzing open market and supermarket contamination prevalence, statistically significant differences were observed in *L. monocytogenes* prevalence in pork and in *Salmonella* prevalence in shrimps (Table 2).

3.1. Salmonella prevalence

Salmonella was detected in 34 (25%) of 136 samples analyzed, including three chicken samples which harbored more than two serotypes. All of 34 isolates were *stn* positive. Supermarket beef (24%), chicken (57%) and pork (12%) had higher contamination prevalence than open markets (0%, 48%, and 0%, respectively). No isolates of *Salmonella* were detected in supermarket shrimp samples. In contrast, 53% of open market shrimp were contaminated. These contaminated shrimps were all derived from the samples collected on the same day, at the same market, but from 10 different vendors. This open market shrimps were sampled twice, and in the second batch, no *Salmonella* was isolated. The high prevalence from one particular sampling date increased the total open market shrimp contamination prevalence. But we suggest that this fact should not be taken as a persistent contamination in the market environment, since the sampling which took place on different day had no *Salmonella* isolates. Supposing that all the booths we have sampled have had the same supplier or utilized the same transport company, probably the contamination may have happened in these stages, and then spread to the vendors.

The World Health Organization (WHO) National *Salmonella* and *Shigella* Centre of Bangkok summarized the notified *Salmonella* serotypes in the Annual Report 2006 (Bangtrakulnonth & Tishyadhigama, 2006). In this report, Stanley, Weltervreden and Corvallis are the most frequently isolated serotypes in raw material foods. Our results are in accordance with Stanley (14%) and Corvallis (14%), but we did not isolate any Weltervreden. In each meat type, the most frequently isolated serotype differed. In open market samples were Corvallis (chicken, 21%) and Stanley (shrimp, 21%), while in supermarket were Rissen (beef, 20%) and Mbandaka (chicken, 20%).

3.2. L. monocytogenes prevalence

In Thailand, it was reported that *L. monocytogenes* was isolated from seafood and vegetables (Swetwiwathana & Chungsamanukool, 1995; Panya & Chamrieng, 1997; Chungsamanokool, Wongsommat, & Phanbualuang, 1995). To our knowledge, this is the first survey of *L. monocytogenes* in retail raw meats in Thailand. *L. monocytogenes* was isolated in 23 (6%) of 388 samples, including one supermarket pork sample which harbored two serotypes. All of 23 isolates were *hly* positive. The contamination prevalence was higher in

Table 2
Prevalence of foodborne pathogens in open markets and supermarkets in Thailand.

Sample	Isolate	Supermarket**	Prevalence (%)	Open market**	Prevalence (%)	*p*-Value
Beef	*L. monocytogenes*	2/68	3	0/40	0	0.529
	E. coli O157	0/46	0	0/33	0	
	Salmonella	6/25	24	0/4	0	1
Chicken	*L. monocytogenes*	1/28	4	5/81	6	1
	E. coli O157	0/17	0	0/44	0	
	Salmonella	4/7	57	13/27	48	1
Pork	*L. monocytogenes*	14/44	32	1/36	3	0.007*
	E. coli O157	0/24	0	0/22	0	
	Salmonella	2/17	12	0/13	0	0.502
Shrimp	*L. monocytogenes*	0/26	0	0/17	0	
	Salmonella	0/26	0	9/17	53	0.001*
	Shigella	0/26	0	0/17	0	
Oyster	*L. monocytogenes*	0/5	0	0/43	0	
	Shigella	0/5	0	0/43	0	
	V. parahaemolyticus	0/5	0	0/43	0	

* Significant at $p < 0.05$ (2-sided) based on comparison between supermarket and open market samples.
** Number of positive samples/total of samples.

supermarket (10%) than in open markets (3%). Supermarket pork (32%) had significantly higher contamination than open market pork (3%). We determined the serotypes of the total 24 isolates, and 16 were 4b and 8 were 4c. No other serotype was found in this collection.

From the 23 samples that tested positive for *L. monocytogenes*, 16 were isolated from pork, among which, 10 were serotype 4b. *L. monocytogenes* has been reported as the pathogen with the highest severity score among zoonoses transmitted by pork in Europe (Fosse, Seegers, & Magras, 2008). The prevalence of *L. monocytogenes* in pork found in the literature varies from 0.7% and 2% in carcasses (Lindblad, Lindmark, Lambertz, & Lindqvist, 2007; Yeh, Chen, & Lin, 2005) to 11.1% and 38% in ready-to-eat food (Cabedo, Picart i Barrot, & Teixido i Canelles, 2008; Berzins, Horman, Lunden, & Korkeala, 2007). Although the serotypes most frequently isolated from pork are 1/2a, 1/2c, and 1/2b (Giovannacci et al., 1999; Thevenot et al., 2006) and 4b has been less frequently isolated from food (Thevenot et al., 2006), most of our isolates were 4b from pork, and this serotype is known to be associated with major outbreaks (Swaminathan & Gerner-Smidt, 2007). Since low virulence 4b has been reported (Roche et al., 2005), there is a need to better analyze these food isolates in their genetic diversity perspective, in order to correlate with human isolates. Nevertheless, the finding of 4b in this collection is a matter of concern and further characterization regarding virulence would be needed for risk assessment.

3.3. E. coli O157

The 186 samples analyzed for *E. coli* O157 tested negative. However, two suspected isolates were mauve colonies on CHROMagar O157 TAM agar plates and agglutinated with O157 antiserum. We have screened for *stx*, $rfbE_{O157}$, and $fliC_{H7}$ genes by PCR, in order to assure that these colonies were not results of cross-reaction (Bettelheim, Evangelidis, Pearce, Sowers, & Strockbine, 1993; Aleksic, Karch, & Bockemuhl, 1992; Voravuthikunchai, Keisaku, Iida, & Honda, 2002). All the PCR results were negative, concluding that these isolates were not *E. coli* O157. To date, *Stx* positive O157 from food has been reported in Thailand only by Vuddhakul, Patararungrong et al. (2000). Chomvarin et al. (2005) has obtained O157 from ready-to-eat food, but non-virulent strain, and Suthienkul et al. (1990) has reported *stx* isolation from retail meat, but no O157 was found, while Voravuthikunchai et al. (2002) did not detect any O157. In addition, no case of diarrhea associated with *E. coli* O157 has been reported (Kalnauwakul, Phengmak, Kongmuang, Nakaguchi, & Nishibuchi, 2007; Ratchtrachenchai, Subpasu, Hayashi, & Ba-Thein, 2004; Leelaporn et al., 2003). The reason of the non-identification of *E. coli* O157 either in food or in clinical cases has been discussed rigorously (Voravuthikunchai, Chaowana, Perepat, Iida, & Honda, 2005).

3.4. Shigella

An outbreak of *Shigella dysenteriae* associated with consumption of coconut milk dessert has been reported in Thailand (Hoge, Bodhidatta, Tungtaem, & Echeverria, 1995), and more recently, Denmark and Australia have reported outbreaks of *S. Sonnei* associated with baby corn imported from Thailand (Lewis et al., 2007). Although culture-confirmed *Shigella* incidence isolated from patients with diarrhea in Thailand is 0.6/1000 population per year (von Seidlein et al., 2006), we did not isolate any *Shigella* in this study. Suspected colonies on selective agars were confirmed as negative in subsequent tests either by biochemical testes or also by PCR screening.

3.5. V. parahaemolyticus

Among 48 oyster samples tested for *V. parahaemolyticus*, three open market samples yielded typical green colonies on TCBS agar plates. When these isolates were analyzed in Nutrient Broth with 0%, 3%, 8% and 10% of NaCl, they only grew in 3% NaCl. A typical *V. parahaemolyticus* would grow in 3% and 8% of NaCl. In addition, when analyzed by PCR, none of the isolates harbored *tdh*, thermostable direct hemolysin, a gene related to pathogenicity. In previous reports, *tdh* negative strains have been frequently isolated from food, and *tdh* positive strains more often from clinical isolates (Serichantalergs et al., 2007; Vuddhakul, Chowdhury, et al., 2000; Wong, Chen, Liu, & Liu, 1999; Yamamoto et al., 2008). Although *tdh*-positive *V. parahaemolyticus* was not isolated in this study, seasonal factor may have influenced the results.

3.6. Salmonella antibiotic resistance

In the antibiotic susceptibility test, most of the *Salmonella* isolates showed resistance to sulfisoxazole, tetracycline and streptomycin (Table 3). Multidrug-resistant *Salmonella* (resistant to four or more antibiotics) were found both in supermarket and open market isolates. The serotypes resistant to the largest number of antibiotics among supermarket samples were Rissen and Stanley (isolated from beef), showing resistance to six of nine antibiotics tested, while among open market samples, Schwarzengrund (isolated from chicken) was resistant to eight antibiotics. None of

Table 3
Salmonella isolates antibiotic resistance profile and serotypes among meat samples from open markets and supermarkets in Thailand ($n = 77$).

Market	Resistance profile	Meat type	Serotype (n)
Open market	Susceptible	Chicken	Bareilly (1)
	Susceptible	Shrimp	Stanley (1)
	Sul	Chicken	Mbandaka (1), Corvallis (3)
	Sul	Shrimp	Bareilly (1), Stanley (3)
	SulAmp	Chicken	Mbandaka (1)
	SulNal	Chicken	Corvallis (3)
	SulTet	Chicken	Bareilly (1), Corvallis (2)
	SulStr	Chicken	Corvallis (1)
	SulStr	Shrimp	Bareilly (3), Derby (1), Stanley (6)
	SulAmpStr	Chicken	Agona (1)
	SulTetAmp	Chicken	Agona (1), Bareilly (3)
	SulStrGen	Chicken	Bareilly (1), Mbandaka (1)
	SulTetNal	Chicken	Corvallis (1), Typhimurium (1)
	SulTetStr	Chicken	Hato (1)
	SulTetStr	Shrimp	Stanley (1)
	SulTetAmpStr	Chicken	Anatum (1), Kentucky (1)
	SulTetAmpNal	Chicken	Krefeld (1), Mbandaka (1)
	SulAmpNalCip	Chicken	Krefeld (1)
	SulTetAmpStr	Chicken	Bareilly (1), Saintpaul (1)
	SulTetAmpStrGen	Chicken	Anatum (1)
	SulTetAmpNalChl	Chicken	Albany (1)
	SulTetAmpNalCip	Chicken	Krefeld (2)
	SulTetAmpStrNalChl	Chicken	Albany (1)
	SulTetAmpStrNalCip	Chicken	Krefeld (1)
	SulTetAmpStrNalKan	Chicken	Mbandaka (1)
	SulTetAmpStrNalGenCip	Chicken	Krefeld (1)
	SulTetAmpStrNalGenChlKan	Chicken	Schwarzengrund (1)
Supermarket	Sul	Chicken	Mbandaka (1)
	Tet	Chicken	Mbandaka (1)
	Tet	Pork	Senftenberg (1)
	SulTet	Chicken	Menden (1), Mbandaka (2)
	SulTetStr	Chicken	Mbandaka (1)
	SulTetAmp	Beef	Rissen (1)
	SulTetStr	Beef	Rissen (1)
	SulAmpStrNal	Chicken	Albany (1)
	SulTetAmpStr	Pork	Stanley (1)
	SulTetAmpNal	Beef	Rissen (1)
	SulTetAmpStr	Beef	Anatum (1), Rissen (2)
	SulTetAmpStrChl	Chicken	Rissen (1)
	SulTetAmpStrNal	Pork	Stanley (1)
	SulTetAmpStrNal	Beef	Vejle (1)
	SulTetAmpStrGen	Beef	Rissen (1)
	SulAmpStrGenKan	Beef	Corvallis (1)
	SulTetAmpStrGenChl	Beef	Rissen (1), Stanley (1)

Gen (Gentamycin 10 μg), Sul (Sulfisoxazole 25 μg), Chl (Chloramphenicol 30 μg), Cip (Ciprofloxacin 5 μg), Kan (Kanamycin 30 μg), Nal (Nalidixic Acid 30 μg), Tet (Tetracycline 30 μg), Amp (Ampicillin 10 μg), Str (Streptomycin 10 μg).

shrimp isolates were multidrug-resistant. Although it is a single isolate, in this study Schwarzengrund showed resistance to eight of nine antibiotics tested. It is a matter of concern because it is reported that human *Salmonella* infection caused by Schwarzengrund in Thailand has increased from 0% in 1992 to 2.4% in 2001 (Aarestrup et al., 2007). Considering the increased incidence of this serotype, added to its multidrug-resistance, the probability of complicated Schwarzengrund infection may increase.

3.7. L. monocytogenes antibiotic resistance

In the *L. monocytogenes* antibiotic susceptibility test, all the isolates showed resistance to penicillin, except for one. Open market isolates showed resistance to ampicillin, clindamycin and penicillin, while supermarket isolates showed resistance also to ciprofloxacin, gentamicin, streptomycin and trimethoprim-sulfamethoxazole. Among pork isolates with serotype 4b, there was an isolate resistant to ampicillin, penicillin, gentamicin, and trimethoprim-sulfamethoxazole (Table 4). These antibiotics are commonly used for treatment of listeriosis, and the existence of multidrug-resistant isolates emphasizes the need for sensitivity test for treatment of foodborne disease.

Occurrence of multidrug-resistant strains of bacteria has increased in frequency in the past few years. When added to the fact that the genetic elements can be exchanged between intestinal bacteria (Callaway, Edrington, Anderson, Byrd, & Nisbet, 2008), they threaten high risk population lives, such as newborn and immunocompromised persons. In Thailand, bacteremia among HIV-infected patients, have been reported to range between 20% and 30%, and the most isolated pathogen is *Salmonella* (Mootsikapun, 2007; Srifuengfung, Chokephaibulkit, Yungyuen, & Tribuddharat, 2005). Among bacteremia caused by non-typhoidal salmonellosis in Thailand, up to 30% were multidrug-resistant (Kiratisin, 2008). Although the mortality rate caused by salmonellosis had decreased in the past years, the increase of multidrug-resistant strains may lead to a therapeutic failure. Although listeriosis is relatively rare in HIV-infected persons, listerial central nervous system infections in immunocompromised patients may have severe clinical forms (Eckburg, Montoya, & Vosti, 2001; Guerra et al., 2004). Furthermore, an ampicillin-resistant *L. monocytogenes* causing brain abscess has been reported in Thailand (Treebupachatsakul, Srifeungfung, & Chayakulkeeree, 2006). This study detected the serotype 4b frequently associated with outbreaks, and the finding of isolate resistant to commonly used antibiotics for listeriosis therapy, may indicate a risk for the consumers.

Except for *Salmonella* contamination in shrimps, supermarkets contamination prevalence for *Salmonella* and *L. monocytogenes* were higher than open markets. Although there is a tendency to think that open markets are more susceptible to cross-contaminations than supermarkets due to constant exposure to environmental factors such as dust, rodents and insects, if the products displayed at the open markets are received directly from the slaughter house, and sold out at the end of the selling day, possibly the freshness may be the reason for low contamination. In addition, depending on the supermarket cutting process and storage conditions, once the contaminated meat is introduced in the environment, several packages may become contaminated, and due to time duration between the cutting and the consumption, the pathogen such as *L. monocytogenes* which grows in refrigeration temperature may increase. Furthermore, the multidrug-resistant isolates are a public health concern, since they may lead to a therapeutic failure.

Table 4
L. monocytogenes isolates antibiotic resistance profile and serotypes among meat samples from open markets and supermarkets in Thailand ($n = 24$).

Market	Resistance profile	Meat type	Serotype (n)
Open market	Pen	Chicken	4b (1)
	PenAmp	Chicken	4b (1)
	PenCliAmp	Pork	4b (1)
	PenCliAmp	Chicken	4b (2), 4c (2)
Supermarket	Amp	Pork	4c (1)
	Pen	Chicken	4b (1)
	Pen	Pork	4b (1), 4c(1)
	PenCli	Pork	4c (2)
	PenAmp	Beef	4b (1)
	PenAmp	Pork	4b (1), 4c (2)
	PenCliAmp	Pork	4b (5)
	PenCliAmpCip	Pork	4b (1)
	PenCliAmpCipGenStrSxt	Pork	4b (1)

Amp (Ampicillin 10 μg), Cli (Clindamycin 2 μg), Cip (Ciprofloxacin 5 μg), Pen (Penicillin 10 μg), Gen (Gentamycin 10 μg), Str (Streptomycin 10 μg), Stx (Trimethoprim-sulfamethoxazole 23.75/1.25 μg).

Acknowledgements

We thank Mr. Krittitee Krittapardmetakul for his collaboration in collecting part of *L. monocytogenes* isolates. This work was supported by a grant from Ministry of Education, Culture, Science and Technology (MEXT), Formation Program of Research Center for Emerging and Re-emerging Infectious Diseases, Promotion of Research on Emerging and Re-emerging Infectious Diseases, by a Grant-in-Aid for Scientific Research (1625503) from the Japan Society for the Promotion of Science (JSPS), by a grant from the Ministry of Health, Labor, and Welfare (Research of Food Safety).

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