ANTIMICROBIAL RESISTANCE AND MOLECULAR CHARACTERIZATION OF *SALMONELLA ENTERICA* SEROVAR KEDOUGOU ISOLATES FROM CLINICAL SPECIMENS AND ENVIRONMENTAL SAMPLES IN THAILAND, 2006-2009

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Abstract. *Salmonella enterica* serovar Kedougou was among the top 10 serovars causing salmonellosis in humans and animals during 2006-2009 in Thailand. Two hundred and twenty-four *S.* Kedougou isolates from human, food and environmental samples were collected from 2006 to 2009. Antimicrobial susceptibility of all isolates, presence or absence of antimicrobial resistance genes and pulsed-field gel electrophoresis (PFGE) profiles were determined. Multidrug resistance (resistance to at least three different classes of antimicrobials) was observed in 126/185 (68.1%) and 31/39 (79.5%) of *S*. Kedougou isolates from human and environmental origins, respectively. Nine (4%) *S*. Kedougou isolates were positive for extended spectrum β-lactamase (ESBL), including 8 isolates from humans and 1 isolate from raw food. Seven of 8 ESBL-producing isolates from humans harbored $bla_{\text{CTX-M-63}}$ and $bla_{\text{TEM-1b}}$, and the remaining isolate $bla_{\text{CMY-2}}$ and $bla_{\text{CTX-M-63}}$. Additionally, one ESBL-producing isolate from fresh pork harbored only $bla_{\text{CMY-2}}$. All ESBL-producing isolates positive for $bla_{\text{CTX-M-63}}$ were resistant to cefotaxime. The PFGE clonal characteristics among the isolates from human and environmental sources may indicate a recent spread of this serovar. The presence of multidrug resistance and β-lactamase genes in *S*. Kedougou isolated from humans and raw food poses a potential public health problem in Thailand.

Keywords: *Salmonella* Kedougou, antimicrobial susceptibility, β-lactamase genes, extended spectrum β-lactamase

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INTRODUCTION

Nontyphoidal *Salmonella* strains are among the most common cause of human gastroenteritis worldwide (Helms *et al*, 2004; Voetsch *et al*, 2004). More than 2,600 serovars of *Salmonella enterica* have been

described (Guibourdenche *et al, 2010*). The two dominant serovars isolated from clinical specimens are *S.* Typhimurium and and *S.* Enteritidis (Madigan *et al*, 2006), which are the causative agents of most cases of salmonellosis worldwide, but such infection is self-limiting (Galanis *et al,* 2006). The majority of human cases of salmonellosis in industrialized countries are caused by the consumption of contaminated food from animal origin (Soumet *et al*, 1999). However, in developing countries, salmonellosis can be caused by contamination in the entire food chain (animal feed, living animal, slaughterhouses, retail sector, and restaurant) in addition to human-to-human transmission (Bertrand *et al,* 2010).

S. Kedougou infection can lead not only to illness but it can also develop drug resistance, leading to possible widespread complications as the organism can infect both animal and human hosts, and drug resistance will result in longer and more difficult courses of treatment. Studies have shown that the prevalence of multidrug-resistant *S.* Kedougou is increasing (Pornruangwong *et al*, 2012). In 2007, a hospital in Algeria isolated *S.* Kedougou producing extended-spectrum β-lactamases (ESBLs) (Touati *et al*, 2008). Multidrug-resistant *S.* Kedougou containing class 1 integron and *Salmonella* genomic islands was reported in Thailand (Khemtong and Chuanchuen, 2008). Recently, *S.* Kedougou was discovered to demonstrate resistance to third-generation cephalosporins and fluoroquinolones commonly used in the treatment of salmonellosis (Pornruangwong *et al*, 2012). Resistance to β-lactams in *S. enterica* is primarily due to the production of acquired β-lactamases (Michael *et al*, 2006). Among these, TEM and OXA-1 are the enzymes most frequently associated with ampicillin and amoxicillin/clavulanate resistance (Güerri *et al*, 2004). Resistance of *Salmonella* to third-generation cephalosporins is mediated by the production of ESBLs of the TEM, SHV and CTX-M lineages, all of which are associated with different mobile genetic elements (Güerri *et al*, 2004). ESBLs have been described not only in clinical *Salmonella* isolates but also in isolates from animals and food (Riaño *et al*, 2009).

Thus, there is a possibility of multidrug-resistant *S.* Kedougou becoming a public health problem in Thailand. In the occurrence of 2008, the antimicrobial susceptibilities and pulsed-field gel-electrophoresis (PFGE) profiles of *S.* Kedougou isolates from human salmonellosis in Thailand and Denmark were reported (Pornruangwong *et al*, 2012). This is the first report of antimicrobial resistance profiles and ESBL resistance genes antimicrobial *S.* Kedougou isolates from human, animal, food, and environmental samples collected in Thailand during 2006-2009.

MATERIALS AND METHODS

Salmonella strains

A total of 224 *S.* Kedougou isolates from different sources, such as human clinical cases, food, animals and environmental swabs collected from 2006 to 2009 were obtained from the National WHO Salmonella and Shigella Center, Nonthaburi, Thailand. *S.* Kedougou isolates from humans (n = 185) and the environment including food, animal reservoirs and environmental swabs (n = 39) were randomly selected for antimicrobial susceptibility testing. Isolates were confirmed as *S.* Kedougou according to antigenic characterization based on Kauffmann-White scheme (Poppof, 2001).

Table 1
PCR primers used to identify ESBL genes of *Salmonella* Kedougou.

Gene	Sequence (5′ to 3′)	T_{Anneal} (°C)	Amplicon size	Reference
bla_{TEM}	F: GCGGAACCCCTATTTG R: ACCAATGCTTAATCAGTGAG	50	964	Olesen *et al* (2004)
bla_{SHV}	F: TTCGCCTGTGTATTATCTCCCTG R: TTAGCGTTGCCAGTGYTCG	55	854	Hasman *et al* (2005)
bla_{CMY-2}	F: GCACTTAGCCACCTATACGGCAG R: GCTTTTCAAGAATGCGCCAGG	55	758	Hasman *et al* (2005)
bla_{ACC}	F: AGCCTCAGCAGCCGGTTAC R: GAAGCCGTTAGTTGATCCGG	55	818	Hasman *et al* (2005)
bla_{OXA-1}	F: ATGAAAAACACAATACATATCAACTTCGC R: GTGTGTTTAGAATGGTGATCGCATT	55	820	Olesen *et al* (2004)
bla_{OXA-2}	F: ACGATAGTTGTGGCAGACGAAC R: ATYCTGTTTGGCGTATCRATATTC	55	602	Hasman *et al* (2005)
bla_{CTX-M}	F: ATGTGCAGYACCAGTAARGTKATGGC R: TGGGTRAARTARGTSACCAGAAYCAGCGG	55	593	Miro *et al* (2002)

K = G/T; R = A/G; Y = C/T.

Antimicrobial susceptibility testing and ESBL assay

Disk diffusion tests were performed according to the Clinical and Laboratory Standards Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards) recommendations (CLSI, 2008) using disks (Oxoid, Hampshire, England) impregnated with ampicillin (AMP; 10 μg), amoxicillin/clavulanic acid (AMC; 30 μg), cefotaxime (CTX; 30 μg), ceftriaxone (CRO; 30 μg), ceftazidime (CAZ; 30 μg), cefpodoxime (CPD; 30 μg), ciprofloxacin (CIP; 5 μg), chloramphenicol (CHL; 30 μg), streptomycin (STR; 10 μg), nalidixic acid (NAL; 30 μg), norfloxacin (NOR; 10 μg), trimethoprim-sulfamethoxazole (SXT; 25 μg), and tetracycline (TET; 30 μg). Mueller-Hinton agar (Oxoid) was used for culture and zones of growth inhibition were measured following incubation for 24 hours at 37°C. *Escherichia coli* ATCC 25922 was employed as quality control and was tested under the same antimicrobial testing conditions. The method described by CLSI (2008) for "other Enterobacteriaceae" was used to perform double-disk diffusion for the screening of ESBL-producing strains. Double-disk diffusion was performed using cefotaxime and cefotaxime/clavulanic acid combination disks (Oxoid, Hampshire, England). *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 was used as positive and negative control strain, respectively.

PCR-based antimicrobial resistance genes characterization

Genomic DNA from cultures grown at 37°C on Mueller-Hinton agar containing antimicrobials was extracted using a Wizard® Genomic DNA Purification Kit (Promega, Madison, WI). The presence of genes implicated in resistance to β-lactams and cephalosporin (bla_{TEM}, bla_{ACC}, bla_{SHV}, bla_{OXA}, bla_{CMY} and bla_{CTX-M}) were detected using PCR. Primers and amplification

conditions are listed in Table 1. Ex*Taq* DNA polymerase (Takara Bio, Shiga, Japan) was used in all PCR experiments. Thermocycling conditions for all reactions were as follows: 95°C for 5 minutes; followed by 35 cycles of 98°C for 10 seconds, T_{Anneal} °C (Table 1) for 30 seconds, and 72°C for 60 seconds. Amplicons were analyzed by 1% agarose gel- electrophoresis at 100 V for 1 hour. Bands were stained with ethidium bromide and observed under UV light. Gel-purified amplicons were sequenced by Macrogen (Seoul, South Korea). DNA sequences were compared to those at GenBank database using BLAST search program.

Pulsed-field gel electrophoresis (PFGE)

*Xba*I PFGE was performed according to the United States Centers for Disease Control and Prevention PulseNet protocol (Ribot *et al*, 2016). *Salmonella* Braenderup H9812 served as control strain. In brief, a PFGE plug was prepared by transferring bacterial cells into 2 ml of cell suspension buffer (CSB; 100 mM Tris pH 8.0 and 100 mM EDTA). Concentration of cell suspension was adjusted to A_{610nm} of 0.4-0.5. Then, 0.4 ml aliquot of cell suspension was added with 20 µl of 20 mM proteinase K and 0.4 ml of melted 1% Seakem Gold agarose (New England Biolabs, Ipswich, MA) containing 1% SDS. The mixture was dispensed into the plug mold and allowed to solidify. The plug was incubated with 5 ml of cell lysis buffer (50 mM Tris pH 8.0, 50 mM EDTA, 1% sarcosyl, and 0.1 mg/ml proteinase K) at 54°C for 3 hours with constant agitation (170 rpm). The plug sample was washed two times with 15 ml of reagent grade type water and four times with TE buffer (pre-heated to 50°C). *Xba*I (TOYOBO, Osaka, Japan) was used for DNA digestion at 37°C for 7 hours. The plug was subjected to PFGE using the CHEF-DR® III system (Bio-Rad, Hercules, CA), with an initial switch time of 2.2 seconds, a final switch time of 63.8 seconds, an included angle of 120°, and a running time of 21 hours at 6 volts. The gel was stained with ethidium bromide solution for 45 minutes and results were recorded using a gel documentation system (Syngene, Cambridge, UK). The PFGE patterns were analyzed using BioNumerics software package (version 4.0; Applied Maths, Kortrijk, Belgium). Tolerance was determined according to the value when all H9812 patterns obtained with the same electrophoretic parameters were indistinguishable. PFGE patterns were compared and clustered using Dice's coefficient with 1.5% position tolerance and an optimization of 1.5%. Dendrogram was constructed using an unweighted-pair group method with average linkage (UPGMA) (Sneath and Sokal, 1973).

RESULTS

Occurrence of *S.* Kedougou

A total of 19,224 *Salmonella* positive samples were isolated from patients and other sources in Thailand during 2006-2010, of which *S*. Kedougou was serotyped in 308/14,886 (2.1%) isolates and 162/3868 (4.2%) of the overall nontyphoid *Salmonella* from human and other samples, respectively. *S*. Kedougou ranked 10th in the overall nontyphoid *Salmonella* serovars from human specimens in 2006 and 2010. The highest incidence of this serovar in human infection was 2.7% in 2006, followed by 2.5% in 2008 and 1.0% in 2009 (Table 2). *S*. Kedougou accounted for 3.3% of the *Salmonella* isolates from food and environmental samples in 2006, followed by an increase to 5% in 2007 (Table 2).

Antimicrobial susceptibility of *S.* Kedougou isolates

Antimicrobial susceptibilities of 224

Table 2
Proportion of *S.* Kedougou found in human and other sources (food, animal and environmental swabs) in Thailand, 2006-2010.

Year	Human		Other sources	
	Salmonella Kedougou Number (%)	Nontyphoid *Salmonella* Number (%)	*Salmonella* Kedougou Number (%)	Nontyphoid *Salmonella* Number (%)
2006	80 (2.7)	2,931 (97.3)	8 (3.3)	244 (96.7)
2007	69 (2.5)	2,720 (97.5)	22 (5.0)	438 (95.0)
2008	76 (2.5)	3083 (97.5)	8 (2.0)	402 (98.0)
2009	29 (1.0)	2,986 (99.0)	48 (4.0)	1194 (96.0)
2010	54 (1.7)	3,166 (98.3)	76 (4.8)	1,590 (95.2)
Total	308 (2.1)	14,886 (97.9)	162 (4.2)	3,868 (95.8)

Table 3
Antibiotic susceptibilities of *S.* Kedougou isolates from humans (n = 185) and other samples (food, animal and environmental swabs) (n = 39) in Thailand, 2006-2009.

Antibiotics	Human (%)			Other sources (%)		
	S	I	R	S	I	R
AMP	53 (29)	1 (<1)	131 (71)	7 (18)	0 (0)	32 (82)
AMC	170 (92)	3 (2)	12 (6)	34 (87)	3 (8)	2 (5)
CTX	175 (95)	2 (1)	8 (4)	38 (97)	0 (0)	1 (3)
CAZ	177 (96)	8 (4)	0 (0)	38 (97)	0 (0)	1 (3)
CRO	177 (96)	0 (0)	8 (4)	38 (97)	0 (0)	1 (3)
CPD	177 (96)	0 (0)	8 (4)	38 (97)	0 (0)	1 (3)
CHL	32 (17)	9 (5)	144 (78)	4 (10)	1 (3)	34 (87)
STR	22 (12)	138 (75)	25 (13)	10 (25.6)	19 (49)	10 (26)
TET	16 (9)	4 (2)	165 (89)	2 (5)	2 (5)	35 (90)
SXT	147 (79.5)	1 (<1)	37 (20)	34 (87)	1 (3)	4 (10)
NAL	167 (90)	16 (9)	2 (1)	39 (100)	0 (0)	0 (0)
CIP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NOR	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

S, susceptibility; I, moderate susceptibility; R resistant. AMP, amoxicillin; AMC, amoxicillin + clavulanate; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; CPD, cefpodoxime; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; NAL, nalidixic acid; CHL, chloramphenicol; NOR, norfloxacin.

S. Kedougou isolates from humans (n = 185) and environmental samples (food, animal and environmental swabs) (n = 39) revealed multidrug resistance (resistance to at least three different classes of antimicrobials) was observed in 126/185 (68%) and 31/39 (79%) of *S.* Kedougou isolates from human and environmental origins, respectively (Table 3). *S.* Kedougou from human isolates demonstrated highest

Table 4
Multidrug resistance patterns and β-lactamase genes of *S.* Kedougou isolates from clinical and other sources in Thailand, 2006-2009.

Lab ID/Year	Source	Specimen	Antimicrobial resistance pattern	β-Lactamase gene	ESBL
SH1409/06	Human	Stool	AMP,AMC,CHL,CTX,CAZ,CPD,CRO,TET	TEM, CTX-M-63	+
SO365/06	Environmental swab	Butcher	AMP,CHL,TET	TEM	-
SO898/06	Raw material foods	Pork	AMP,CHL,TET	TEM	-
SO903/06	Ready-to-eat foods	Pork	AMP,CHL,STR,SXT,TET	TEM	-
SH1703/07	Human	Rectal swab	AMP,AMC,CTX,CPD,CRO,CHL,SXT,TET	TEM, CTX-M-63	+
SH455/07	Human	Pus	AMP,AMC,CTX,CPD,CRO,CHL,TET	TEM, CTX-M-63	+
SH297/07	Human	Rectal swab	AMP,AMC,CTX,CPD,CRO,CHL,TET	TEM, CTX-M-63	+
SH608/08*	Human	Stool	AMP,AMC,CHL,CTX,CAZ,CPD,CRO,STR,SXT,TET	CMY-2, CTX-M-63	+
SH1757/08*	Human	Rectal swab	AMP,AMC,CHL,CTX,CAZ,CPD,CRO,TET	TEM, CTX-M-63	+
SH2445/08*	Human	Stool	AMP,AMC,CHL,CTX,CAZ,CPD,CRO,STR,SXT,TET	TEM, CTX-M-63	+
SH1466/09	Human	Stool	AMP,AMC,CTX,CPD,CRO,CHL,SXT,TET	TEM, CTX-M-63	+
SO471/09	Raw material food	Pork	AMP,AMC,CHL,CTX,CAZ,CPD,CRO,STR,SXT,TET	CMY-2	+

AMP, amoxicillin; AMC, amoxicillin + clavulanate; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; CPD, cefpodoxime; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; NAL, nalidixic acid; CHL, chloramphenicol; NOR, norfloxacin.
* Antimicrobial susceptibility from Pornruangwong *et al* (2012).

resistance to tetracycline (89%), chloramphenicol (78%) and ampicillin (71%). All *S*. Kedougou isolates from food and environmental sources showed reduced susceptibility to 10 antibiotics with the exception of ciprofloxacin, nalidixic acid and norfloxacin. These isolates were most resistant to ampicillin (82%), chloramphenicol (87%) and tetracycline (90%). All isolates from human and nonhuman sources were susceptible to fluoroquinolones (ciprofloxacin and norfloxacin).

ESBL-producing isolates detection and characterization of β-lactam resistance genes

Eight out of 224 (4%) *S*. Kedougou isolates from humans were positive for ESBLs production, with 2, 4 and 2 isolates resistant to 7 (AMP, AMC, CHL, NAL, STR, SXT, and TET), 8 (AMP, AMC, CTX, CPD, CRO, CHL, SXT, TET or AMP, AMC, CHL, CTX, CAZ, CPD, CRO, and TET) and 10 (AMP, AMC, CHL, CTX, CAZ, CPD, CRO, STR, SXT, and TET) antimicrobial agents, respectively (Table 4). All ESBL-containing isolates were resistant to ampicillin and amoxicillin/clavulanate; cross resistance to cefotaxime, ceftriaxone and ceftriaxone occurred in 100% of isolates, and cross resistance to ceftazidime in 50% of isolates. Additionally, all ESBL-producing isolates were resistant to chloramphenicol and tetracycline. Seven of eight ampicillin and cefotaxime resistant isolates from humans had positive results for both $bla_{\text{CTX-M}}$ and bla_{TEM} (Table 4); however, only one isolates (SH608/08) had previously been reported to have both $bla_{\text{CMY-2}}$ and $bla_{\text{TEM-1b}}$ (Pornruangwong *et al*, 2012). Additionally, one ESBL-producing isolate from fresh pork (SO471/09) was positive for only $bla_{\text{CMY-2}}$. All $bla_{\text{CTX-M}}$ and bla_{TEM} amplicons were confirmed as $bla_{\text{CTX-M-63}}$ and $bla_{\text{TEM-1b}}$ by DNA sequencing.

PFGE analysis

Nearly indistinguishable PFGE patterns were present in isolates from Thai patients, food and environmental samples obtained from different regions of Thailand during 2006-2009 (Fig 1). PFGE of isolates SH608, SH1178, SH1757 and SH2445 have previously been reported by a Pornruangwong *et al* (2012). One ESBL-producing isolates (SH1757/08) and two non-ESBL-producing isolates (SH529/06 and SH1905/06) collected from human samples shared similar *Xba*I patterns with one isolate (SO182/08) recovered from ready-to-eat food, sharing a similarity of more than of 98% according to the similarity index. However, SO182/08 isolate did not show resistance to third-generation cephalosporins and did not contain ESBL resistance genes. In addition, clinical isolate (SH1403/06) exhibited an identical profile to isolate (SO903/06) collected from ready-to-eat food.

DISCUSSION

To the best of our knowledge, this is the first epidemiological study using both molecular profiling and antimicrobial susceptibility data carried out in *S. enterica* serovar Kedougou from human and environmental samples (food, animals and environment) in Thailand. In a 2008 survey, antimicrobial susceptibility and PFGE of *S*. Kedougou isolates from human salmonellosis in Thailand and Denmark was reported (Pornruangwong *et al*, 2012). *S*. Kedougou is the most common serovar found in fresh pork and pork food products in Thailand between 2002 and 2010, compared with other nontyphoid *Salmonell*a serovars. In a previous survey from 1993 to 2002, this serovar was rarely detected (Bangtrakulnonth *et al*, 2004); however, an increasing proportion

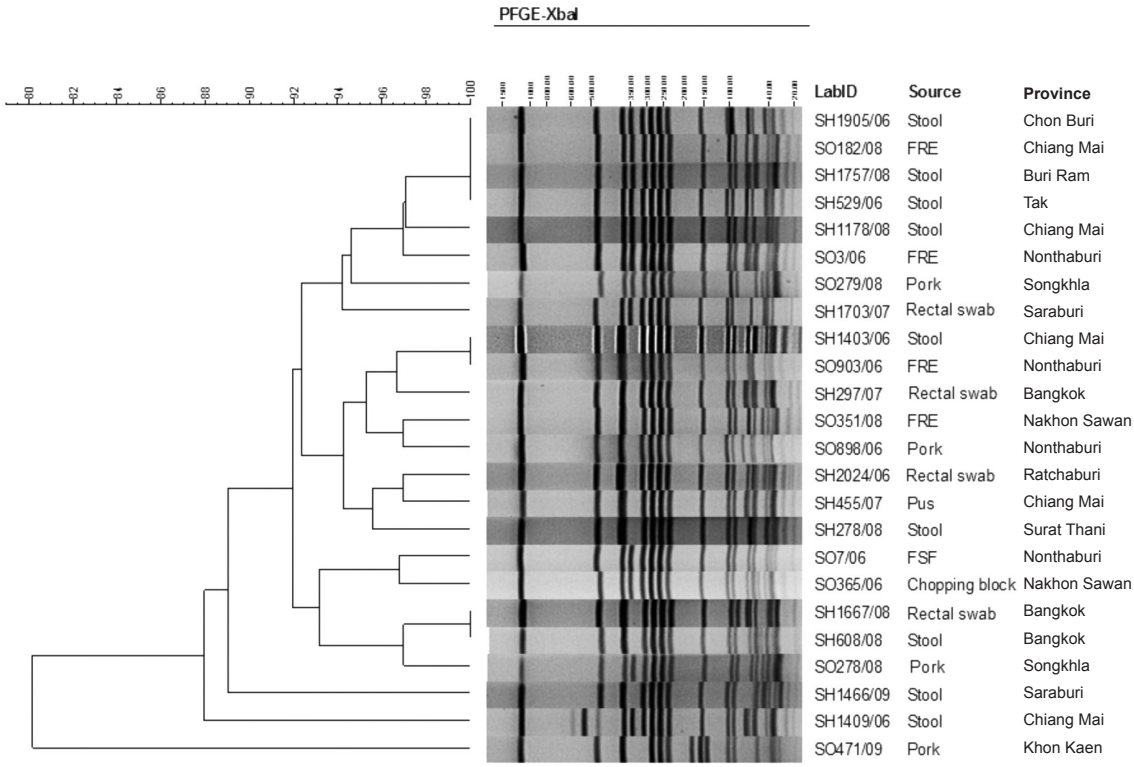

Fig 1–PFGE dendrogram of *Salmonella* Kedougou isolates from human (n = 14) and environmental samples (raw food, animal and other sources) (n = 10) in Thailand, 2006-2009. *Xba*I-digested DNA (37°C for 7 hours) was subjected to PFGE using the CHEF-DR® III system (Biorad), with an initial switch time of 2.2 seconds, a final switch time of 63.8 seconds, an included angle of 120°, and a running time of 21 hours at 6 volts. Dendrogram was constructed using an unweighted-pair group method with average linkage method. Scale bar denoted percent identity. FRE, ready-to-eat food; FSF, frozen sea food.

of *S*. Kedougou in patients and food was observed during 2006-2010 signifying an emerging problem.

Multidrug-resistant *S*. Kedougou may pose a risk to humans, especially if the bacteria are resistant to quinolones and third-generation cephalosporins. Previous studies conducted in Thailand have reported the presence of multidrug resistant *Salmonella* from a wide range of serovars (Archambault *et al*, 2006; Hendriksen *et al*, 2008; Khemtong and Chuanchuen, 2008; Chuanchuen and Padungtod, 2009; Sirichote *et al*, 2010). This study observed that eight ESBL-producing *S*. Kedougou isolates from human were resistant to third-generation cephalosporins, namely, cefotaxime, ceftriaxone and cefpodoxime, drugs of choice for the treatment of *Salmonella* infection in humans. Seven of the isolates harbored $bla_{\text{CTX-M-63}}$ and $bla_{\text{TEM-1b}}$, and the remaining isolate contained $bla_{\text{CMY-2}}$ and $bla_{\text{CTX-M-63}}$. $bla_{\text{CTX-M-63}}$ is an uncommon variant belonging to a $bla_{\text{CTX-M-8}}$ subgroup

previously described in *E. coli* (Hopkins *et al*, 2006). Furthermore, to the best of our knowledge, this is the first report identifying a *S*. Kedougou strain isolated from raw food harboring bla_{CMY-2}.

In spite of data regarding antimicrobial usage for disease prevention, enrofloxacin and ceftiofur, third-generation cephalosporins, still are extensively used in swine production because these growth promoting compounds are not not prohibited in Thailand (Kulwichit *et al*, 2007). The use of these antimicrobials on farm may contribute to the evolution of drug resistant clones. Although the data from this study did not suggest that *S*. Kedougou is an invasive serovar, if resistance to third-generation cephalosporins is acquired, the treatment of this infection using β-lactam antibiotics could be severely compromised. The reduction of antibiotic susceptibility to third-generation cephalosporins observed in isolates from humans and fresh pork might be linked to the use of β-lactam antibiotics in swine farms.

When *S*. Kedougou isolates from food and animal sources in Thailand were subtyped by PFGE, a cluster of human and animal isolates was revealed, indicating a relationship among these isolates. Of note, an isolate of pork origin had an identical PFGE profile to those of ESBL and non-ESBL producing isolates from human specimens. The incidence of *S*. Kedougou originating from pork source has been on the rise in Thailand during the past few years (Hendriksen *et al*, 2009) as it is one of the 10 most common serovars isolated from pork in the country (Vindigni *et al*, 2007).

In summary, this study shows a high frequency of multidrug resistance among *S*. Kedougou isolated from humans, food and animal sources in Thailand, posing a potential public health threat, especially from *S*. Kedougou isolates that are resistant to third-generation cephalosporins. This study also identifies possible routes of transmission via the food chain. PFGE typing suggests that *S*. Kedougou from humans and raw foods collected in different location in Thailand were clonally related, which may indicate that this serovar has recently spread and that resistance has evolved locally among the isolates in Thailand.

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REFERENCES

Archambault M, Petrov P, Hendriksen RS, *et al.* Molecular characterization and occurrence of extended-spectrum betalactamase resistance genes among *Salmonella enterica* serovar Corvallis from Thailand, Bulgaria, and Denmark. *Microb Drug Resist* 2006; 12: 192-8.

Bangtrakulnonth A, Pornreongwong S, Pulsrikarn C, *et al. Salmonella* serovars from humans and other sources in Thailand, 1993-2002. *Emerg Infect Dis* 2004; 10: 131-6.

Bertrand S, Dierick K, Heylen K, *et al.* Lessons learned from the management of a national outbreak of *Salmonella* Ohio linked to pork meat processing and distribution. *J Food Prot* 2010; 73: 529-34.

Chuanchuen R, Padungtod P. Antimicrobial resistance genes in *Salmonella enterica* isolates from poultry and swine in Thailand. *J Vet Med Sci* 2009; 71: 1349-55.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved Standard-3rd ed. M31-A3.

Wayne: CLSI, 2008.

Galanis E, Lo Fo Wong D M, *et al.* Web-based surveillance and global *Salmonella* distribution, 2000-2002. *Emerg Infect Dis* 2006; 12: 381-8.

Güerri ML, Aladueña A, Echeita A, Rotger R. Detection of integrons and antibiotic-resistance genes in *Salmonella enterica* serovar Typhimurium isolates with resistance to ampicillin and variable susceptibility to amoxicillin-clavulanate. *Int J Antimicrob Agents* 2004; 24: 327-33.

Guibourdenche M, Roggentin P, Mikoleit M, *et al.* Supplement 2003-2007 (No. 47) to the White-Kauffmann-Le Minor scheme. *Res Microbiol* 2010; 161: 26-9.

Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. beta-Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. *J Antimicrob Chemother* 2005; 56: 115-21.

Helms M, Simonsen J, Molbak K. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium. *J Infect Dis* 2004; 190: 1652-4.

Hendriksen RS, Bangtrakulnonth A, Pulsrikarn C, *et al.* Risk factors and epidemiology of the ten most common *Salmonella* serovars from patients in Thailand: 2002-2007. *Foodborne Pathog Dis* 2009; 6: 1009-19.

Hopkins KL, Deheer-Graham A, Threlfall EJ, Batchelor MJ, Liebana E. Novel plasmid-mediated CTX-M-8 subgroup extended-spectrum beta-lactamase (CTX-M-40) isolated in the UK. *Int J Antimicrob Agents* 2006; 27: 572-5.

Khemtong S, Chuanchuen R. Class 1 integrons and *Salmonella* genomic island 1 among *Salmonella enterica* isolated from poultry and swine. *Microb Drug Resist* 2008; 14: 65-70.

Kulwichit W, Chatsuwan T, Unhasuta C, Pulsrikarn C, Bangtrakulnonth A, Chongthaleong A. Drug-resistant nontyphoidal *Salmonella* bacteremia, Thailand. *Emerg Infect Dis* 2007; 13: 501-2.

Madigan S, Moran G, Pederson DR. Unresolved states of mind, disorganized attachment relationships, and disrupted mother-infant interactions of adolescent mothers and their infants. *Dev Psychol* 2006; 42: 293-304.

Michael GB, Butaye P, Cloeckaert A, Schwarz S. Genes and mutations conferring antimicrobial resistance in *Salmonella*: an update. *Microbes Infect* 2006; 8: 1898-914.

Miro E, Navarro F, Mirelis B, *et al*. Prevalence of clinical isolates of *Escherichia coli* producing inhibitor-resistant beta-lactamases at a university hospital in Barcelona, Spain, over a 3-year period. *J Antimicrob Chemother* 2002; 46: 3991-4.

Olesen I, Hasman H, Aarestrup FM. Prevalence of β-lactamases among ampicillin resistant *Escherichia coli* and *Salmonella* isolated from food animals in Denmark. *Microb Drug Resist* 2004; 10: 334-40.

Popoff MY. Antigenic formulas of the *Salmonella* serovars. 8th revision. Paris: WHO Collaborating Centre for Reference and Research on *Salmonella*, Institute Pasteur, 2001.

Pornruangwong S, Hendriksen RS, Pulsrikarn C, *et al.* Epidemiological investigation of *Salmonella enterica* serovar Kedougou in Thailand. *Foodborne Pathog Dis* 2012; 8: 203-11.

Riaño I, García-Campello M, Sáenz Y, *et al.* Occurrence of extended-spectrum β-lactamase-producing *Salmonella enterica* in northern Spain with evidence of CTX-M-9 clonal spread among animals and humans. *Clin Microbiol Infect* 2009; 15: 292-5.

Ribot EM, Fair MA, Gautom R, *et al.* Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathog Dis* 2006; 3: 59-67.

Sirichote P, Hasman H, Pulsrikarn C, *et al.* Molecular characterization of extended spectrum cephalosporinases (ESC) producing *Salmonella* Choleraesuis from patients in

Thailand and Denmark. *J Clin Microbiol* 2010; 48: 883-8.

Sneath PHA, Sokal RR. Numerical taxonomy: the principles and practice of numerical classification. San Francisco: WH Freeman, 1973.

Soumet C, Ermel G, Rose N, *et al.* Evaluation of a multiplex PCR assay for simultaneouns identification of *Salmonella* spp, *Salmonella* Enteritidis and *Salmonella* Typhimurium from environmental swabs of poultry houses. *Lett Appl Microbio* 1999; 28: 113-7.

Touati A, Benallaoua S, Gharout A, *et al.* First report of CTX-M-15 in *Salmonella enterica* serotype Kedougou recovered from an Algerian hospital. *Pediatr Infect Dis J* 2008; 27: 479-80.

Vindigni SM, Srijan A, Wongstitwilairoong B, *et al.* Prevalence of foodborne microorganisms in retail foods in Thailand. *Foodborne Pathog Dis* 2007; 4: 208-15.

Voetsch AC, Van Gilder TJ, Angulo FJ, *et al.* FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. *Clin Infect Dis* 2004; 38 (Suppl 3): S127-34.