THE EPIDEMIOLOGICAL RELATIONSHIP BETWEEN *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM AND *SALMONELLA ENTERICA* SEROVAR 4,[5],12:I:- ISOLATES FROM HUMANS AND SWINE IN THAILAND

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Abstract. A total of 138 isolates of *S*. Typhimurium and *S*. 4,[5],12:i:- from humans and swine in Thailand during 2003-2006, were evaluated for antimicrobial sensitivity by the disk diffusion method against 10 antimicrobial drugs and pulsed-field gel electrophoresis (PFGE) with endonuclease *Xba*I to investigate the epidemiological relationship among isolates. It was found that all isolates were classified into 27 antimicrobial resistance patterns, and 80% of *S*. Typhimurium and 95.4% of *S*. 4,[5],12:i:- isolates were resistant to three or more antimicrobial agents. By PFGE testing, the 84 PFGE patterns were categorized into A to Z patterns. Eighty percent of *S*. Typhimurium and 71.3% of *S*. 4,[5],12:i:- isolates in 7 major PFGE patterns had close clonal relationships (≥85% similarity). Our studies indicate the spread of genetically identical clones of *S*. Typhimurium and *S*. 4,[5],12:i:- in humans and swine in Thailand.

INTRODUCTION

Salmonellosis is one of the most common and widely distributed foodborne diseases. Among the over 2,400 serovars identified within *Salmonella enterica* subsp. *enterica*, *S*. *enterica* serovar Typhimurium continues to be one of the most frequently recovered from food animals worldwide (Herikstad *et al*, 2002; Zhao *et al*, 2005).

In Thailand, *S*. Typhimurium and *S*. 4,[5], 12:i:- have been identified in samples from human and non-human (food animals) origin over the last few decades. *S*. 4,[5],12:i:- has also been found among the top five *Salmonella* species isolated from human cases of food-borne salmonellosis in addition to *S*. Typhimurium (Bangtrakulnonth *et al*, 2004). *S*. 4,[5],12:i:- is an atypical emergent monophasic phase serovar which resembles *S*. Typhimurium except that it has no phase 2 flagellar antigen. Some studies have been carried out in order to determine the genetic relationship between these serotypes and to confirm the hypotheses that *S*. 4,[5],12:i:- is a monophasic variant of *S*. Typhimurium (Echeita *et al*, 1999; 2001; Agasan *et al*, 2002; Amavisit *et al*, 2005). *S*. 4,[5],12:i:- has become the most frequently encountered serovar in swine and the second most frequently encountered serovar in pork products (Duffy *et al*, 2001; Ferris *et al*, 2002). In the last few years in Thailand, *S*. Typhimurium and *S*.

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4,[5],12:i:- have developed increased nalidixic acid resistance in isolates from human foodborne illness and septicemia cases. Many countries have reported that non-typhoidal *Salmonella* isolates collected from clinical specimens were resistant to quinolones and fluoroquinolone, such as nalidixic acid, norfloxacin, ciprofloxacin, and enrofloxacin (Hakanen *et al*, 1999).

The rapid increase in the frequency of occurrence of *S*. 4,[5],12:i:- has made necessary further studies in order to determine its origin and its genetic relationship with *S*. Typhimurium. The objective of this study was to determine the genetic relationship between *S*. Typhimurium and *S*. 4,[5],12:i:- isolates from humans and swine in Thailand during 2003-2006. The isolates were compared by pulsed-field gel electrophoresis (PFGE) and antimicrobial resistance patterns for ten antimicrobial drugs.

MATERIALS AND METHODS

Salmonella Typhimurium and *Salmonella* 4,[5],12:i:- isolates

A total of 138 isolates of *S*. Typhimurium (*n*=30) and *S*. 4,[5],12:i:- (*n*=108) obtained from patients with foodborne illness or septicemia and swine from slaughterhouses, pork from local markets and swine feces in farms between 2003-2006 were used in this study. Twenty-six isolates of *S*. Typhimurium were isolated from humans (6 isolates from blood, 7 isolates from rectal swabs, and 13 isolates from stool) and 4 isolates from swine (2 fecal isolates from farms and 2 isolates of pork from local markets). Seventy-three isolates of *S*. 4,[5],12:i:- were isolated from humans (25 isolates from blood, 19 isolates from rectal swabs, and 29 isolates from stool) and 35 isolates from swine (15 isolates from carcasses in slaughterhouses, 9 fecal isolates from farms, and 11 isolates from pork at local markets).

All isolates were sent to the WHO National *Salmonella* and *Shigella* Center for Thailand for conventional biochemical and serological testing. The biochemical tests performed were those described by Ewing (1986). Serotyping of *Salmonella* isolates was performed on the basis of somatic O and phase 1 and phase 2 flagellar antigens by agglutination tests with antisera (S & A Reagents Lab, Thailand) according to the Kauffman-White Scheme (Popoff and Le Minor, 2001).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method of the National Committee for Clinical Laboratory Standard (NCCLS) (National Committee for Clinical Laboratory Standard, 2000). Ten antimicrobial agents in the form of disks were employed for susceptibility testing of 138 *Salmonella* isolates. The concentrations of the antimicrobial agents were as follows:- amoxicillin-clavulanic acid (AMC) 30 μg, ampicillin (AMP) l0 μg, chloramphenicol (C) 30 μg, cefotaxime (CTX) 30 μg, ciprofloxacin (CIP) 5 μg, nalidixic acid (NA) 30 μg, norfloxacin (NOR) l0 μg, streptomycin (S) 30 μg, sulfamethoxazole-trimethoprim (SXT) 25 μg, and tetracycline (T) 30 μg. In this test, *Escherichia coli* ATCC 25922 was used as the quality control strain.

Pulse field gel electrophoresis (PFGE)

PFGE macrorestriction analysis was performed in accordance with the PulseNet Protocal (Centers for Disease Control and Prevention, 2001). Briefly, cells of *Salmonella* serovars were lysed, and the genomic DNA was embedded in agarose plugs. The DNA was digested in the agarose by using the restriction enzyme *Xba*I. The restriction fragments were separated with a CHEF-DRIII (Bio-Rad) apparatus with the following reagents and conditions : 1% Seakem Gold agarose (Biolabs, England), 0.5X Tris-borate-EDTA, 14ºC, and 6 V/cm for 19 hours, with switch times ranging from 2.2 to 63.8 seconds. The

isolate *Salmonella* serovar Braenderup H9812 was used as a reference marker. The gel was stained with ethidium bromide for 30 minutes, destained two times for 20 minutes each with distilled water. The gel image was captured using Gel Doc 2000 (Biolabs, England), and converted to a tif file. PFGE profiles were analysed by using Bionumerics software version 3.0 (Biolabs, England). Clustering of patterns was performed by the unweighted pair group method with arithmatic averaging (UPGMA). The relationship between different PFGE profiles was analyzed according to the criteria established by Tenover *et al* (1995).

RESULTS

Microbial susceptibility testing

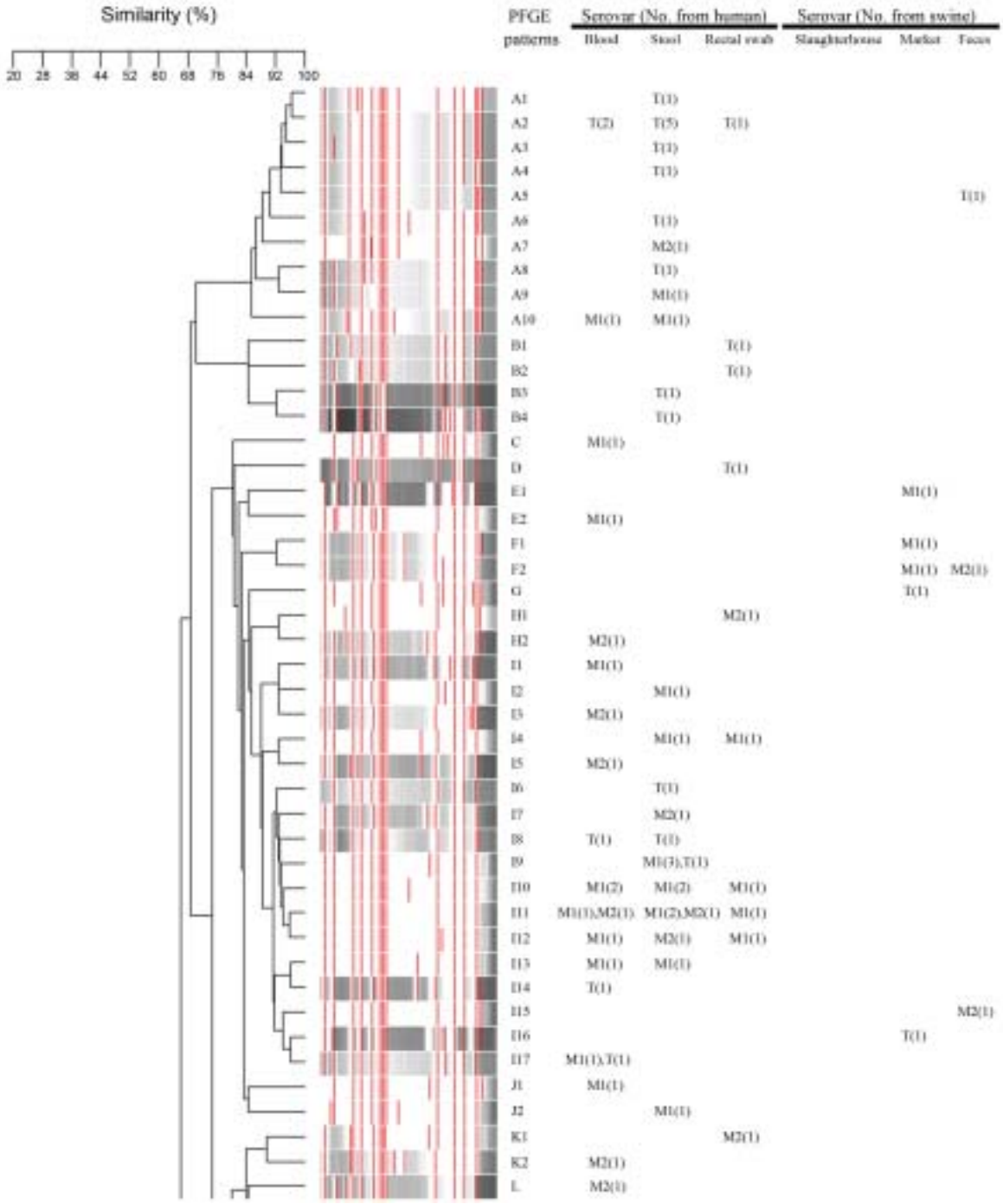
One hundred thirty-eight isolates of *S*. Typhimurium and *S*. 4,[5],12:i:- from human and swine sources were tested with 10 antimicrobial agents (Table 1). All isolates were resistant to nalidixic acid. Thirty isolates of *S*. Typhimurium from humans and swine were susceptible to amoxicillin-clavulanic acid, norfloxacin and ciprofloxacin. *S*. Typhimurium isolates exhibited resistance to tetracycline (73.3%), sulfamethoxazole-trimethoprim (60.0%), ampicillin (53.3%) and streptomycin (30.0%) and to a lesser extent to chloramphenicol (13.3%) and cefotaxime (6.7%). *S.* 4,[5],12:i:- was more resistant to ampicillin (92.6%), tetracycline (80.5%), sulfamethoxazole-trimethoprim (71.3%), streptomycin (65.7%) and chloramphenicol (33.3%). One (0.9%) isolate from blood and 4 (3.7%) isolates from pork in local markets exhibited intermediate resistance to ciprofloxacin and norfloxacin, respectively. Multiresistance (resistance to three or more antimicrobial agents) was found 80% (24/30) and 95.4% (103/108) in *S*. Typhimurium and *S.* 4,[5],12:i:- isolates, respectively.

PFGE patterns of *S.* Typhimurium and *S.* 4,[5],12:i:- from humans and swine

Eighty-four PFGE patterns were identified among the 138 *S*. Typhimurium and *S*. 4,[5],12:i:- isolates digested with *Xba*I, which were clustered into 7 major patterns (A1-A10, E1-E2, F1-F2, I1-I17, M1-M8, P1-P6, R1-R12) with >85% similarity PFGE patterns (Fig 1). PFGE patterns A1-A10 were found in *S*. Typhimurium from humans and swine. The PFGE patterns E1 and E2 were only found in *S*. 4,[5],12:i:- isolates from human blood and pork. The PFGE patterns F1 and F2 were found in *S*. 4,[5],12:i:- isolates from swine feces from the farm. The dominant PFGE patterns I1-I17 were found in *S*. Typhimurium isolates from human stool and pork isolates from the local market and *S*. 4,[5],12:i:- isolates from human and swine feces. The PFGE patterns M1-M8 were observed in *S*. Typhimurium and *S*. 4,[5],12:i:- isolates from humans and isolates from carcasses in slaughterhouses. The PFGE patterns P1-P6 and R1-R12 were found only in *S*. 4,[5],12:i:- isolates from humans and pork in a local market, swine from slaughterhouses and in swine feces.

The relation of antimicrobial resistance patterns and PFGE patterns

Antimicrobial resistance of *S*. Typhimurium and *S*. 4,[5],12:i:- from humans and swine were divided into 27 patterns (Table 2). Seventy-five (54.3%) isolates were presented in 3 predominant antimicrobial resistance patterns (including patterns 8, 21, and 25). PFGE patterns A1, A2, and A6 had antimicrobial resistance patterns 8, of which isolates were recovered from only humans. PFGE patterns I1, I3, I4, I5, I7, I8, I9, I10, I11, I12, I13, I14, I15, and I17 had antimicrobial resistance patterns 21 and 25, of which 21 isolates were *S*. 4,[5],12:i:- and 3 isolates were *S*. Typhimurium. PFGE patterns M1, M4, M5, and M7 had antimicrobial resistance pattern 21, of which 3 isolates were *S*. 4,[5],12:i:- and 1 isolate was *S*. Typhimurium. In *S*. 4,[5],12:i:-, 2 isolates had PFGE patterns R1 and R3 with antimicrobial resistance pattern 12.


Similarity (%)
20 28 36 44 52 60 68 76 84 92 100
PFGE patterns
Serovar (No. from human)
Blood
Stool
Rectal swab
Serovar (No. from swine)
Slaughterhouse
Market
Feces
A1 T(1)
A2 T(2) T(5) T(1)
A3 T(1)
A4 T(1)
A5 T(1)
A6 T(1)
A7 M2(1)
A8 T(1)
A9 M1(1)
A10 M1(1) M1(1)
B1 T(1)
B2 T(1)
B3 T(1)
B4 T(1)
C M1(1)
D T(1)
E1 M1(1)
E2 M1(1)
F1 M1(1)
F2 M1(1) M2(1)
G T(1)
H1 M2(1)
H2 M2(1)
I1 M1(1)
I2 M1(1)
I3 M2(1)
I4 M1(1) M1(1)
I5 M2(1)
I6 T(1)
I7 M2(1)
I8 T(1) T(1)
I9 M1(3),T(1)
I10 M1(2) M1(2) M1(1)
I11 M1(1),M2(1) M1(2),M2(1) M1(1)
I12 M1(1) M2(1) M1(1)
I13 M1(1) M1(1)
I14 T(1)
I15 M2(1)
I16 T(1)
I17 M1(1),T(1)
J1 M1(1)
J2 M1(1)
K1 M2(1)
K2 M2(1)
L M2(1)

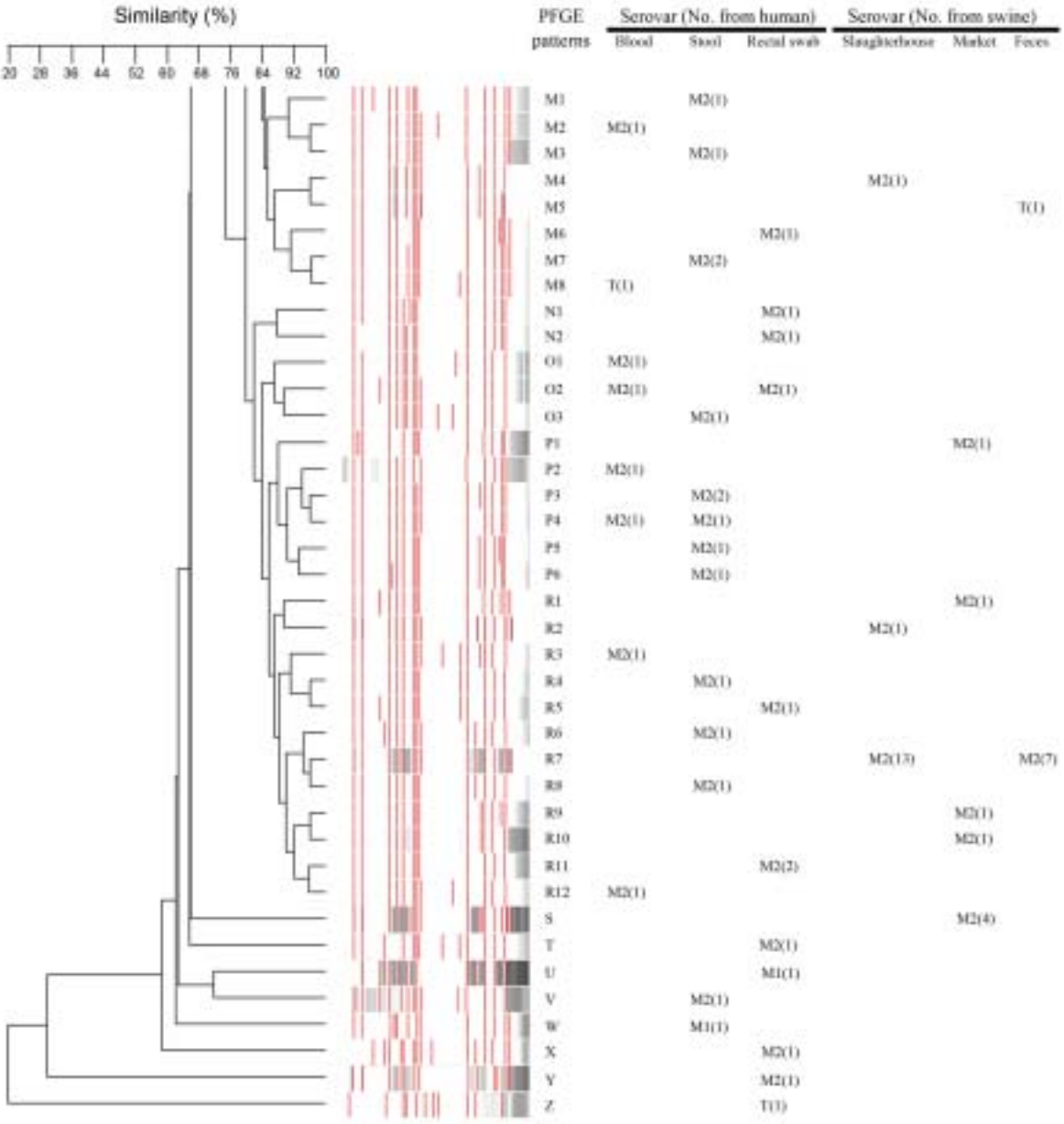


Fig 1–Dendrogram and 84 PFGE patterns of *Xba*I-digested chromosomal DNA of *S.* Typhimurium (*n*=30) and *S.* 4,[5],12:i:- (*n*=108) isolates from humans and swine. PFGE patterns are designated A to Z. T, *S.* Typhimurium; M1, *S.* 4,[5],12:i:-; M2, *S.* 4,[5[,12:i:-

Tabel1

Antimicrobial resistance in *S.* Typhimurium (*n*=30) and *S.* 4,[5],12:i:- (*n*=108) from humans and swine.

Serovar /Source (specimens type)	No. isolates	No. of resistance isolates (%)									
		AMP	AUG	C	CIP	CTX	NA	NOR	S	SXT	T
S. Typhimurium/Humans											
Blood	6	3	0	3	0	0	6	0	3	5	5
Rectal swab	7	3	0	0	0	0	7	0	3	3	3
Stool	13	6	0	1	0	2	13	0	1	7	12
S. Typhimurium/Swine											
Slaughterhouse	-	-	-	-	-	-	-	-	-	-	-
Local market	2	2	0	0	0	0	2	0	0	2	0
Feces	2	1	0	0	0	0	2	0	2	1	2
Total	30	16	0	4	0	0	30	0	9	18	22
		(53.3)	(0.0)	(13.3)	(0.0)	(6.7)	(100)	(0.0)	(30.0)	(60.0)	(73.3)
S. 4,[5],12:i:-/Humans											
Blood	25	23	3	10	1[a]	0	25	0	21	22	19
Rectal swab	19	18	1	8	0	0	19	0	18	18	13
Stool	29	25	0	18	0	1	29	0	25	23	24
S. 4,[5],12:i:-/Swine											
Slaughterhouse	15	15	0	0	0	0	15	0	1	1	15
Local market	10	9	0	0	0	0	10	4[a]	4	10	6
Feces	10	10	0	0	0	0	10	0	2	3	10
Total	108	100	4	36	1[a]	1	108	4[a]	71	77	87
		(92.6)	(3.7)	(33.3)	(0.9)	(0.9)	(100)	(3.7)	(65.7)	(71.3)	(80.5)

[a]The isolates exhibited intermediate resistance.

Table 2
The relation of antimicrobial resistance patterns and PFGE patterns in *S*. Typhimurium and *S*. 4,[5],12:i:- from different sources.

Patterns	Antimicrobial resistance	No. of isolates	PFGE patterns	
			Human	Swine
1	NA	4	A7,D,I17,Z	
2	AMP,NA	3	B1,I 6,I 13	
3	CTX,NA	1	I 2	
4	NA,SXT	1		F1
5	NA,T	2	A2,M3	
6	AMP,NA,SXT	6	T	F2,G,E1,I16,R10
7	AMP,NA,SXT,NOR[a]	1		R9
8	AMP,NA,T	26	A1,A2,A6,I10	R2,R7
9	NA,S,T	2	B2	A5
10	NA,SXT,T	8	A2,A3,A4,A8,B3	
11	AMP,C,NA,S	2	H2,U	
12	AMP,NA,S,SXT	5	H1,I 9,M6,M7,Y	
13	AMP,NA,SXT,T	4	R3,K2	P1,R1,
14	C,NA,S,T	1	O2	
15	C,NA,SXT,T	1	A9	
16	CTX,NA,SXT,T	1	A2	
17	NA,S,SXT,T	2	M2,R4	
18	AMP,C,NA,S,T	2	I11	
19	AMP,C,NA,S,SXT	7	C,J1,K1,L,O2,R5,W	
20	AMP,C,NA,SXT T,	1		F2
21	AMP,NA,S,SXT,T	23	I1,I 5,I 8,I 9,I 13,I 17,M1, M7,N1,N2,P2,P3,P4,R11,X	M4,M5,S
22	AMP,NA,S,SXT,T,CIP[a]	1	R12	
23	AMP,NA,S,SXT,T,NOR[a]	3		S
24	AMP,AUG,NA,S,SXT,T	2	A10,I10	
25	AMP,C,NA,S,SXT,T	26	E2,I 3,I 4,I 7,I 8,I 10,I 11,I 12,I 14, J2,M8,O3,P4,P5,P6,R6,R8,V	I15
26	AMP,AUG,C,NA,S,SXT,T	2	I10,O1	
27	AMP,C,CTX,NA,S,SXT,T	1	B4	

[a]The isolates exhibited intermediate resistance.

DISCUSSION

Over the past decade, *Salmonella* serovar Typhimurium was one of the top five serovars among human isolates most frequently isolated in Thailand (Bangtrakulnonth *et al*, 2004). In 1996 in Thailand, a new serovar with the antigenic formula 4,[5],12:i:- emerged, and since then has been increasing in frequency. This serovar has a high frequency in humans, especially with septicemia, and is also associated with contaminated swine and pork products. In this study, 80% (24/30) of serovar Typhimurium and 97.2% (105/108) of serovar 4,[5],12:i:- exhibited resistance to three or more antimicrobial agents. Echeita *et al* (1999, 2001) found that serovar 4,[5],12:i:- strains had multiresistance to ampicillin, chloramphenicol,

streptomycin, sulfamethoxazole, tetracycline, gentamicin and sulfamethoxazoletrimethoprim, as well as a multidrug resistance pattern found mostly among serovar Typhimurium DT104 strains (Guerra *et al*, 2001). In this study, we found that all isolates of serovar Typhimurium and serovar 4,[5],12:i:- exhibited resistance to nalidixic acid. In the last few years in Thailand, *S*. Typhimurium and *S*. 4,[5],12:i:- have had increasing nalidixic acid resistance in isolates from human food-borne illness and septicemic cases. In our laboratory, 53.1% (264 of 497) of *S*. Typhimurium and *S*. 4,[5],12:i:- showed resistance to nalidixic acid during 2002-2005 from human specimens (data not shown). Many researchers have found that resistance to nalidixic acid in isolates of *Salmonella* is regarded as an indicator of decreased susceptibility to ciprofloxacin (Hakanen *et al*, 1999).

DNA-based strain typing by pulsed-field gel electrophoresis (PFGE) for *Salmonella* serovars is the best method of studying the source of transmission of *Salmonella* serovars and have been subsequently linked to human illness from exposure to animals or contaminanted food animals (Geraldine *et al*, 2005). These studies found similar PFGE patterns in human and swine isolates (close clonal relationship with 85% patterns) (Fig 1). We suggest that serovar 4,[5],12:i:- and Typhimurium were transmitted from retail pork to humans, swine feces to humans, and swine feces to pork. Contaminated pork products by *Salmonella* often are the result of feces being spread to the carcass during slaughting and processing of swine (Wonderling *et al*, 2003). Garaizar *et al* (2002) reported that serovar 4,[5],12:i:- showed a close clonal relationship to serovar Typhimurium by DNA microarray-based typing. A previous study found that serovar 4,5,12:i:- strains lacked the second-phase flagellar antigen encoded for the *fljB* gene. It has been suggested that it could be a monophasic variant of serovar Typhimurium (Echeita *et al*, 2001; Tavechio *et al*, 2004). The specific *mdh* gene of serovar Typhimurium was found in the choromosome of serovar 1,4,[5],12:i:- (Amavisit *et al*, 2005).

These observations found that 24 isolates with PFGE pattern I and 4 isolates with PFGE pattern M shared the same antimicrobial resistance patterns (antimicrobial resistance patterns 21 and 25) and the other 2 isolates in PFGE patterns R1 and R3 of *S*. 4,[5],12:i:- showed antimicrobial resistance pattern 12 in humans and swine. The correlation between PFGE patterns and multidrug resistance may explain the dissemination of *Salmonella* and multidrug resistance isolates in humans, which has been linked to multidrug resistance isolates from pork and food products. This supports the argument of horizontal dissemination of plasmids carrying multidrug resistance genes may be occurring between *Salmonella* in humans and swine. It has been suggested that serovar 4,[5],12:i:- belonged to the serovar Typhimurium phage type DT U302 strains according to PFGE profiles obtained with enzyme *Xba*I and *Bln*I, which shared the same plasmid profile and pattern of multiresistance to antimicrobials from swine samples (de la Torre *et al*, 2003). Therefore, transmission of *Salmonella* to humans can occur via various food vehicles, including meat and produce, and via direct contact with animals and their environment. This study implies that the use of antimicrobial drugs in food animals as well as in humans is an important factor which has resulted in the emergence and persistence of antimicrobial resistance in humans.

REFERENCES

Agasan A, Kornblum J, Williams G. Profile of *Salmonella enterica* subsp. *enterica* (subspecies I) serotype 4,5,12:i:- strains causing food-borne infections in New York City. *J Clin Microbiol* 2002; 40: 1924-9.

Amavisit P, Boonyawiwat W, Bangtrakulnont A. Charaterization of *Salmonella enterica* serovar

Typhimurium and monophasic *Salmonellla* serovar 1,4,[5],12:i:- isolates in Thailand. *J Clin Microbiol* 2005; 43: 2736-40.

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

Centers for Disease Control and Prevention. Standardized molecular subtyping of foodborne bacterial pathogens by pulse-field gel electrophoresis. Atlanta: Centers for Disease Control and Prevention, 2001.

de la Torre E, Zapata D, Tello M, *et al*. Several *Salmonella enterica* subsp. *enterica* serotype 4,5,12:i:- phage types isolated from swine samples originate from serotype Typhimurium DT U302. *J Clin Microbiol* 2003; 41: 2395-400.

Duffy EA, Belk KE, Sofos JN. Bellinger GR, Pape A, Smith GC. Extent of microbial contamination in United State pork retail products. *J Food Prot* 2001; 64: 173-8.

Echeita MA, Aladuena A, Cruchaga S, Usera MA. Emergence and spread of an atypical *Salmonella enterica* subsp *enterica* serotype 4:5,12:i:- strain in Spain. *J Clin Microbiol* 1999; 37: 3425.

Echeita MA, Herrera S, Usera MA. Atypical, flj-negative *Salmonella enterica* subsp. *enterica* strain of serovar 4:5,12:i:- appears to be a monophasic variant of serovar Typhimurium. *J Clin Microbiol* 2001; 39: 2981-3.

Ewing WH. Edwards and Ewing's identification of Enterobacteriaceae. 4th ed. New York: Elsevier Science, 1986.

Ferris KE, Aalsburg AM, Iseminger GR. *Salmonella* serotypes from animals and related sources during July 2001-June 2002. *Proc US Anim Health Assoc* 2002; 106: 467-97.

Garaizar J, Porwollik S, Echeita A, *et al*. DNA microarraybased typing of an atypical monophasic *Salmonella enterica* serovar. *J Clin Microbiol* 2002; 40: 2074-8.

Geraldine D, Dearbhaile M, Colette H, *et al*. Cost-effective application of pulsed-field gel electrophoresis to typing of *Salmonella enterica* serovar Typhimurium. *Appl Environ Microbiol* 2005; 71: 8236-40.

Guerra B, Soto SM, Arguelles JM, Mendoza MC. Multidrug resistance is mediated by large plasmids carrying a class 1 integron in the emergent *Salmonella enterica* serotype [4,[5],12:i:-]. *Antimicrob Agents Chemother* 2001; 45: 1305-8.

Hakanen A, Kotilainen P, Jalava J, Siitonen A, Huovinen P. Detection of decreased fluoroquinolone susceptibility in salmonellas and validation of nalidixic acid Screening test. *J Clin Microbiol* 1999; 37: 3572-7.

Herikstad H, Motarjemi Y, Tauxe RV. *Salmonella* surveillance: a global survey of public health serotyping. *Epidemol Infect* 2002; 129:1-8.

National Committee for Clinical Laboratory Standard. Performance standards for antimicrobial disk susceptibility test: approved standards, 7th ed. Wayne: M2-M7 National Committee for Clinical Laboratory Standard, 2000.

Popoff MY, Le Minor L. Antigenic formulas of the *Salmonella* serovars. 8th ed. Paris: WHO Collaborating Centre for Reference and Reseach on Salmonella, 2001.

Tavechio AT, Fernandes CR, Sueli A. Multiplex PCR identification of the atypical and monophasic *Salmonella enterica* subsp. *enterica* serotype 1,4,[5],12:i:- in Sao Paulo state, Brazil: frequency and antibiotic resistance patterns. *Rev Inst Med Trop S Paulo* 2004; 46: 115-7.

Tenover FC, Arbeit RD, Goering RV, *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233-9.

Wonderling L, Pearce R, Wallace M, *et al*. Use of pulsed-field gel electrophoresis to characterize the heterogenicity and clonality of *Salmonella* isolates obtained from the carcasses and feces of swine at slaughter. *Appl Environ Microbiol* 2003; 69: 4177-82.

Zhao S, Fedorka-Cray PJ, Friedman S, *et al*. Characterization of *Salmonella* Typhimurium of animal origin obtained from the National Antimicrobial Resistance Monitoring System. *Foodborne Pathog Dis* 2005; 2:169-81.