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Spread of antimicrobial resistant *Salmonella* from poultry to humans in Thailand

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Summary (197 words)

Food animal production is important for every country. Several antibiotic agents are used in poultry farming to reduce the economic losses arising from mostly untested, infectious diseases. This continued study was performed to determine the prevalence of antibiotic resistant *Salmonella* in broiler chickens, poultry farmers, and *Salmonella*-bacteremia patients. A total of 121 *Salmonella* isolates were collected from the Thai provinces of Khon Kaen (65 isolates), Ratchaburi (43 isolates), and Phayao (13 isolates). *Salmonella* from chicken showed a high rate of resistance to nalidixic acid and tetracycline. Sixty-four percent of *Salmonella* carried class 1 integron (*intI1* gene positive). Among 121 *Salmonella* isolates, there were 15 serotypes, with Enteritidis the most common. A clonal relationship between the chicken and human isolates was demonstrated by three molecular typing methods: Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction; Pulsed-Field Gel Electrophoresis; and High-Throughput, Multilocus Sequence Typing. A spread of the sequence type 11 (ST11) clone was found between chickens and humans. This study revealed a large-scale *Salmonella* outbreak in Thailand, a link between resistant bacteria from poultry farms and vertical transmission through the food chain, and a horizontal transmission of resistance genes. These results can be used for future surveillance and monitoring.
Introduction (2,964 words)

Bacterial contamination of food and drinking water is the major cause of diarrheal diseases and an occasional cause of serious systemic infections (1, 2). Salmonellosis remains high in most countries using better detection techniques (3), with the food typically involved being eggs, poultry or other meats, and raw milk (4, 5). In Thailand, the incidence of food poisoning is over 120,000 cases/year, and the most common causative agents are *Shigella* spp., *Salmonella* sp. and *Escherichia coli* (6). Therefore, food safety policy is a significant public health issue, and the large-scale food animal production in Thailand requires effective disease management programs (7). The presence of antimicrobial-resistant bacteria in food animals and raw meat products has important public health implications. A previous study revealed the spread of resistant bacteria from supermarkets in Bangkok (8). Knowledge of the genetic background of *Salmonella* isolates in Thailand is limited. The main objectives of this study were to characterize the antibiotic resistance and the causal and clonal relationships of these *Salmonella* from poultry, as well as their identity in patients, using several molecular typing methods.
Materials and methods

Ethics review

The study was approved by the Siriraj Institutional Review Board.

Sample collection

There were 121 Salmonella isolates from cloacal swabs of chickens, rectal swabs of chicken farmers, and blood isolates of patients, with 65 isolates from Khon Kaen, 43 from Ratchaburi, and 13 from Phayao, in 2012–2013. Only 81 isolates could be tested for their phenotype: 40 Salmonella chicken samples of the Ratchaburi Veterinary Research and Development Center only had total DNAs and plasmids for genotypic tests due to an agreement with this site. We additionally included 37 additional Salmonella isolated from bacteremic patients in Bangkok for High-Throughput, Multilocus Sequence Typing.

Bacterial isolation and identification

Salmonella were isolated and identified by the Microbiology Laboratory of the Faculty of Veterinary Medicine, Khon Kaen University, Photharam Hospital, and the Veterinary Research and Development Center (Ratchaburi site). Samples from cloacal swabs (chicken) and rectal swabs (farmers) were incubated at 37°C for 18 hours in buffered peptone water. Then, 0.1ml of pre-enrichment broth were transferred to 10ml of Rappaport–Vassiliadis (RV) broth and incubated at 42°C for 24 hours before plating on to Salmonella Shigella agar and Xylose Lysine Desoxycholate agar. The positive blood culture bottles were streaked on blood agar, chocolate agar, and MacConkey agar. Typical Salmonella colonies were confirmed by standard biochemical tests.

Serological testing for Salmonella

Salmonella serotyping was performed at the Department of Medical Sciences, Ministry of Public Health, Thailand, using panels of Salmonella antisera (S&A Reagents Lab Ltd, Bangkok, Thailand) and the Kauffman–White classification scheme.

Antimicrobial susceptibility testing

Eighty-one Salmonella underwent antimicrobial susceptibility testing (AST) by the disk-diffusion
method on Mueller Hinton agar, following CLSI guidelines. The tested antibiotic disks from BD BBL Sensi-Disc included ampicillin (10µg), amoxicillin/clavulanic acid (20/10µg), chloramphenicol (30µg), ciprofloxacin (5µg), cefotaxime (30µg), nalidixic acid (30µg), norfloxacin (10µg), streptomycin (10µg), tetracycline (30µg), and sulfamethoxazole/trimethoprim (25µg). The inhibition zones of the human and chicken isolates were interpreted using the same CLSI breakpoints (9).

Detection of class 1 integron \((\text{int}\,\text{I})\) and drug resistance genes

PCR amplification was carried out with specific primers for \(\text{int}\,\text{I}\) \((\text{int}\,\text{I}-\text{F}: \text{5}'-\text{GCCTTGCTGTTCTTTCTACGG-3'}, \text{int}\,\text{I}-\text{R}: \text{5}'-\text{GATGCCTGCTTTCTACGG-3'})\) and resistance gene cassettes \((\text{5'CS}: \text{5}'-\text{GGCATCCAAGCAGCAAG-3'}, \text{3'CS}: \text{5}'-\text{AAGCAGACTTGACCTGA-3'})\) (10). Consensus primers for common \(\beta\)-lactamase genes were used as follow; TEM for \(\text{bla}_{\text{TEM}}\) family \((\text{TEM}-\text{F}: \text{5}'-\text{ATGAGTATTCAACATTTCCG-3'}, \text{TEM}-\text{R}: \text{5}'-\text{CTGACAGTTACCAATGCTTA-3'})\) (11), CTX-M for \(\text{bla}_{\text{CTX-M}}\) family \((\text{CTX-M MA1}: \text{5}'-\text{SCSATGTGCAGYACCAGTA-3'}, \text{CTX-M MA2}: \text{5}'-\text{CRATATGRTTGCTGTTG-3'})\) (12) and SHV for \(\text{bla}_{\text{SHV}}\) family \((\text{SHV}-\text{F}: \text{5}'-\text{CACTCAAGGATGTATTGTG-3'}, \text{SHV}-\text{R}: \text{5}'-\text{TTAGCGTTGCCAGTGCTCG-3'})\) (13).

Plasmid replicon typing

Multiplex PCR was adopted for all \(\text{Salmonella}\), using 18 specific primers, for plasmid replicon typing for ones frequently found in \(\text{Enterobacteriaceae}\) (14, 15).

Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR)

The ERIC primers were ERICR1 \((\text{5'-ATGTAAGCTCCTGGGGATTAC-3'})\) and ERIC2 \((\text{5'-AAGTAAGTGACTGGGGTTGACCG-3'})\). PCR amplification was done in 50µl reaction volumes containing nuclease-free water, 1µM of each primer, 0.2mM of dNTP, 1.5mM MgCl₂, 1.5U of DNA polymerase (NEB), 1X supplied buffer, and 1µl (100 ng) of genomic DNA. The cycling profile was initial denaturation \((94\,^\circ\text{C}, \,7\, \text{min})\); 30 cycles of denaturation \((90\,^\circ\text{C}, \,30\, \text{sec})\); annealing \((55\,^\circ\text{C}, \,60\, \text{sec})\);
extension (71°C, 5 min); and final extension (71°C, 16 min). The banding patterns of the isolates were compared for bacterial grouping (16).

**Pulsed-field gel electrophoresis (PFGE)**

A single colony of *Salmonella* isolate was incubated at 37°C for 14–18 h. *XbaI* restriction enzyme was used for DNA digestion as per the standard macro-restriction analysis protocol. PFGE was performed using electrophoresis chamber (CHEF Mapper, BioRad), with an initial switch time of 2.16 sec and a final switch time of 63.8 sec, for 18–19 hours. Lambda was used as the molecular-size marker. The gel was stained with ethidium bromide (1µg/ml) (17).

**High-Throughput, Multilocus Sequence Typing (HiMLST)**

*Salmonella* isolates were typed by the HiMLST method (Roche 454 Sequencing Systems). Briefly, at the first PCR round, MLST housekeeping genes were amplified using primers from standardized MLST schemes with universal tails at 5’end. Heptaplex PCR amplification was done in 10µl reaction volumes containing nuclease-free water, 0.4µM of each primer, 0.2mM of dNTP, 1.8mM MgCl2, 1U of FastStart High Fidelity Reaction Kit (Roche), 1:20 Resolight Dye (Roche), 1X supplied buffer, and 1µl (100ng) of genomic DNA. Amplification was done using LightCycler 480 instrument (Roche). The cycling profile was initial denaturation (95°C, 5 min); 40 cycles of denaturation (95°C, 30 sec); annealing (55°C, 30 sec); and extension (72°C, 60 sec). During the second PCR round, the MLST amplicons were amplified to incorporate 454 sequencing-specific nucleotides and isolate-specific multiplex identifiers (MIDs), a unique DNA barcode for each bacterial isolate, by using fusion primers targeting the universal tails from the first PCR round. Amplification was done using a LightCycler 480 instrument (Roche). The cycling profile was initial denaturation (95°C, 2 min); 35 cycles of denaturation (95°C, 30 sec; annealing (50°C, 30 sec); and extension (72°C, 60 sec). During the first 10 PCR cycles, the annealing was increased by 0.5°C per cycle to 55 °C. Barcoded amplicons were pooled and sequenced in a single-run of high-throughput DNA sequencing technology (Roche 454 GS Junior) (18).
Results

Serological test for *Salmonella*

Of 81 *Salmonella* isolates, 15 serotypes were identified, the most common being *S. Enteritidis*, *S. Typhimurium*, and *S. Schwarzengrund* (Table 1).

Antimicrobial susceptibility test (AST)

No *Salmonella* isolate was completely susceptible to all tested antibiotics. Seventy-four percent (38/51) of chicken isolates were resistant to nalidixic acid, and 63% (12/19) of patient isolates were resistant to nalidixic acid and ampicillin. As to cefotaxime, 26% (5/19) of patient isolates were resistant, whereas all chicken and farmer isolates were susceptible (Table 2). Strains resistant to at least 3 antimicrobial agent classes were detected in 27% (22/81) of *Salmonella* isolates.

Detection of class 1 integron and drug resistance genes

All *Salmonella* were tested for class 1 integron (*intI1*, *blaTEM*, *blaSHV*, and *blaCTX-M*). The positive findings for these resistance-related genes by PCR amplification are in Table 3. Twenty-six percent of *Salmonella* from patient isolates were positive for *blaCTX-M*, and all *blaCTX-M* positive isolates were resistant to cefotaxime. Four patient isolates carrying *blaCTX-M* were *S. Choleraesuis*, which was not found in chicken isolates.

Plasmid replicon typing

Plasmid IncI1 (n = 15) and IncA/C (n = 5) were the common plasmid types in *Salmonella* isolates. Plasmid replicon types IncA/C and IncI1 were found in *Salmonella* from Khon Kaen and Ratchaburi, and IncHI1 was detected in chicken and patient isolates. Distribution of the plasmid replicon types is shown in Table 4.

Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR)

Eighteen major ERIC patterns of *Salmonella* were found. The majority of *Salmonella* were categorized into ERIC_Sal07 (17/121 isolates) and ERIC_Sal08 (17/121) patterns. ERIC_Sal08 (17/121) and ERIC_Sal10 (14/121) were common in chicken, while ERIC_Sal07 (17/121) was found primarily in patients. Based on the serotyping technique, ERIC_Sal03, ERIC_Sal04,
ERIC_Sal07, and ERIC_Sal08 were S. Schwarzengrund, S. Corvallis, S. Enteritidis, and *Salmonella* ser 4, 5, 12:i:-, respectively.

**Pulsed-field gel electrophoresis**

Thirty-two known serotypes of *Salmonella* were tested for *XbaI*-pulsed-field gel electrophoresis. Seven major patterns were observed among *Salmonella* isolates. The predominant pattern, designated pulsotype P3, was found in 4 isolates, while pulsotypes P1, P2, P4, P5, P6, and P7 were observed in 3, 3, 2, 3, 2, and 2 isolates, respectively. The remaining 13 isolates each showed a unique PFGE pattern (singleton). There was heterogeneity in 5 isolates of S. Schwarzengrund (pulsotype P4, 2 isolates, and 3 singletons); likewise, 5 isolates of S. Choleraesuis showed pulsotype P5 (3 isolates) and 2 singletons.

**HiMLST**

Ninety-six *Salmonella* isolates were selected for HiMLST: 18 were from patients, 11 from farmers, and 30 from chickens, and 37 bacteremia isolates came from HIV-infected patients in Bangkok. From GS Junior sequencing, 93 of 96 isolates were successfully sequenced for all 7 standard housekeeping genes. The MLST sequence types (ST) obtained from HiMLST were ST11, ST139, ST96, ST34, ST213, ST469, ST29, ST1549, ST1, ST13, ST19, ST40, ST48, ST64, ST446, and ST516. Common STs in patients were ST11, ST139, and ST34 (serovar *S. Enteritidis*, *S. Choleraesuis*, and *Salmonella* ser. 4, 5, 12:i:-, respectively). Figure 1 maps the ST spread.
Discussion

As *Salmonella* exists in humans and chickens as colonizers or pathogens, we selected isolates from healthy farmers and chickens, and bacteremic patients. In poultry farms, tetracycline is used to promote growth, and nalidixic acid to prevent disease. Hence, the chicken isolates showed a high resistance to tetracycline and nalidixic acid (Table 2). In 1990, the International Agency for Research on Cancer declared chloramphenicol as “probably carcinogenic to humans”. The European Union subsequently banned the use of chloramphenicol in food-animal production (19). However, we found 12% of *Salmonella* from chickens were resistant to chloramphenicol. Florfenicol is commonly used by chicken farms, and this drug may select for chloramphenicol resistance. In 1990, the International Agency for Research on Cancer declared chloramphenicol as “probably carcinogenic to humans”. The European Union subsequently banned the use of chloramphenicol in food-animal production (19). However, we found 12% of *Salmonella* from chickens were resistant to chloramphenicol. Florfenicol is commonly used by chicken farms, and this drug may select for chloramphenicol resistance. In the patient isolates, cephalosporin-resistant isolates were detected, whereas all chicken isolates were not resistant (Table 2), suggesting limited exposure to this antibiotic at poultry farms. Most cephalosporin-resistant *Salmonella* isolates carried *blaCTX-M* (11/12 isolates); 4 of these *Salmonella* from patients were S. Choleraesuis, which was previously reported in swine from Thailand and Taiwan (20). A high incidence of *blaCTX-M* (45/122 isolates) in healthy swine has also been reported (21). A third-generation cephalosporin, ceftiofur, is used extensively for swine production in Thailand (22). Although none of the chicken-farmer isolates were ESBL-producing strains, a high prevalence (58.2%) of *blaCTX-M* ESBL-producers among healthy Thais has been reported (23).

For resistance-gene transfer in bacteria, we investigated the role of horizontal gene transfer via detection of the integron element along with their resistance gene cassettes and plasmids. A high prevalence of *intI1* of class 1 integron was demonstrated in chicken isolates (64%). Another study found the *intI1* gene in 40% of the chicken meat sold at Thai supermarkets (7). The prevalence of *intI1* in *Salmonella* has been reported in Korea (9.1%) (24) and Japan (90%) (25).

For the plasmid study, the common *Salmonella* isolate replicon types were IncI1 and IncA/C. Consistent with an earlier report, no replicon type could be identified in more than 50% of the *Salmonella* isolates (26). We may need whole genome sequencing with a long-read feature to identify the untypeable groups of this study. IncA/C was found in 4 chicken isolates and 1 patient
isolate. The IncA/C was from the broad-host range plasmid family, which has been associated with multi-drug resistant phenotypes among enteric bacteria, including *Salmonella* (27). *Salmonella* PS4, serotype Choleraesuis, showed resistance to chloramphenicol, nalidixic acid, streptomycin, tetracycline, and trimethoprim. In a report from Thailand, *S*. Choleraesuis from human blood culture showed association with *bla*\(_{CMY}\) containing IncA/C (28). Evidence of the circulation of IncA/C in Gram-negative bacteria with their ability to encode resistance genes was elucidated in another study (29). Our finding of IncA/C suggests such plasmids have disseminated in animals and humans. The IncI1 plasmids have been associated with resistance genes, such as *bla*\(_{CTX-M}\) and *bla*\(_{CMY}\), conferring EBSLs and AmpC phenotypes from human and animal origins (30, 31). However, there was no correlation in this study between the IncI1 and EBSLs phenotype of *Salmonella* isolates.

To understand the epidemiological relationship between bacterial strains, serotyping, ERIC-PCR, PFGE, and HiMLST were used to identify and differentiate strains of *Salmonella*. ERIC-PCR and PFGE are considered to have high discriminatory power suitable for short term outbreak investigations, in contrast to serotyping and MLST, which are suitable for long term dissemination studies. *Salmonella* Enteritidis was most commonly found (26%), followed by *S*. Typhimurium (15%) and *S*. Schwarzengrund (12%) in this study. From a previous report, the most common serotypes found in a food animal (frozen chicken meat) in Thailand are *S*. Enteritidis (20%), *S*. Hadar (9.3%), and *S*. Paratyphi B (7.1%) (32). The top 3 serotypes from chicken isolates in our study were *S*. Typhimurium, *S*. Enteritidis, and *S*. Schwarzengrund. *S*. Enteritidis and *S*. Choleraesuis are common serotypes in bacteremic Thai patients (22). In the current study, *S*. Choleraesuis was not detected in chickens or chicken farmers, but we found 5 isolates from patients. The presence of this swine-related serotype is evidence that the consumption of contaminated meat is a risk factor for Salmonellosis.

ERIC-PCR categorized the isolates into 17 major patterns, with ERIC_Sal07 and ERIC_Sal08 the most common. Members of ERIC_Sal08 were *S*. Typhimurium and *Salmonella* ser. 4, 5, 12:i:-, the monophasic variant of *S*. Typhimurium. We had only the purified DNA of ERIC_Sal10 Ratchaburi
samples.

We could not confirm the serotype of the ERIC_Sal10; however, all members of this ERIC pattern were from Ratchaburi, suggesting *Salmonella* ERIC_Sal10 was circulating in this province. The members of ERIC_Sal07 were isolated from different hosts and provinces (chickens from Khon Kaen, and patients from Phayao). All isolates with this ERIC pattern belonged to serotype Enteritidis. Similarly for ERIC_Sal08, the serotype was Typhimurium and its variants. Isolates showing identical ERIC patterns were considered genetically related. These results suggest clonal spreads of *Salmonella* in Thailand, with direct links of specific strains of *Salmonella* in the food chain and patients. ERIC-PCR also showed certain heterogeneity in 3 serotypes: S. Enteritidis (ERIC_Sal07, ERIC_Sal25, and 2 unique isolates); S. Choleraesuis (ERIC_Sal09 and 3 unique groups); and S. Typhimurium (ERIC_Sal08 and ERIC_Sal26). Two S. Anatum isolates demonstrated intra-serotype heterogeneity, which was observed in ERIC_Sal20 and ERIC_Sal21. A similar discrepancy has been reported, probably due to different discriminatory powers of the two typing techniques (33).

From the PFGE analysis of the 32 *Salmonella* isolates, 7 major and 13 singleton XbaI-PFGE patterns were observed, based on serotyping and ERIC-PCR patterns. Pulsotype P3 predominated (HS6, HS7, HS11, and S7 from Khon Kaen). All members of the pulsotypes P1, P3, and P4 had the same serotypes and ERIC patterns within their own pulsotypes. These concordant results from several typing methods suggest the dissemination of the same clones of *Salmonella* among chickens and farmers (Table 5). Pulsotype P5, found in 3 Ratchaburi patients, belonged to serotype Choleraesuis. This finding may reflect a clonal spread of *Salmonella* in bacteremic patients in Ratchaburi. PFGE showed differences between serotypes Typhimurium and *Salmonella* 4, 5, 12:i:-, while ERIC-PCR could not distinguish these Typhimurium variants. For serotype Enteritidis, ERIC-PCR yielded ERIC_Sal07, while PFGE showed 3 singletons, despite the isolate PS1 and PS5 banding patterns being very similar; this demonstrated that, on this occasion, PFGE had a higher discriminatory power. Still, pulsotype P5 of S. Choleraesuis could be grouped into different ERIC-PCR types (ERIC_Sal09, ERIC_Sal41, and ERIC_Sal45).
For the HiMLST study, ninety-three (96%) isolates were successfully STs assigned, as reported by an earlier study (17). *Salmonella* is already colonized in the intestinal system of poultry, which is not normally seen in humans, indicating *Salmonella* from patient’s blood culture in this study is a true pathogen. The most prevalent ST from HiMLST was ST11; all members in ST11 were serotype Enteritidis, consistent with studies on food and clinical specimens in Portugal, Japan, Brazil, and China (34–37). Also, ST34 (*Salmonella* ser.4,5,12:i:-) was shared between chickens, farmers, and patients, suggesting a linkage from chicken farms to the community and subsequently to patients. The relationships of *Salmonella* isolates by serotype, ERIC PCR, PFGE, and HiMLST are shown in Table 5. PFGE and ERIC PCR with high discriminatory powers have shown potential for intra-serotype typing, making them appropriate tools for short-term outbreak investigations. In contrast, MLST can detect nucleotide changes at the DNA level, which are not apparent with serotyping. MLST data are portable and suitable for global and large-scale epidemiological analyses (38). A combination of various typing methods can therefore discriminate between, and pinpoint, the specific strains of *Salmonella* among similar serotypes.

The high prevalence and wide geographic distribution of ST11 in all populations and in all regions of Thailand (Northern, Northeastern, Western, and Central; Figure 1) in this study indicate a large-scale *Salmonella* outbreak in Thailand. All members of ST139 were serotype Choleraesuis and were only found in patients. ST139 is highly pathogenic in humans, usually causing septicemic disease rather than intestinal tract infection (39). The high prevalence of the Choleraesuis serotype in patients is of concern, particularly as swine are its specific host. The spread of this serotype from swine to patients warrants further investigation.

**Conclusions**

Our study showed major clones of Thai *Salmonella* in poultry and humans. The distribution of ST11 in all populations indicates links between poultry and the community. These isolates were multi-drug resistant and could cause diseases in human hosts, posing the risk of unsuccessful treatment. This information is vital to strengthening Thai antimicrobial control policies for farming in order to
reduce selection pressure. Nationwide collaboration is needed to perform surveillance studies that track and control these resistant bacteria and improve food safety.

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Conflicts of interest: All authors declare that there are no conflicts of interest related to this study.
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Figure 1. The distribution of MLST sequence types of *Salmonella* isolates in 4 provinces. S = chicken isolates; HS = farmer isolates; PS = patient isolates.
Table 1. The origin and frequency of *Salmonella* serotypes in this study.

| Serotype                              | Chickens | Farmers | Patients | Total |
|---------------------------------------|----------|---------|----------|-------|
| S. Enteritidis                        | 11       | 0       | 10       | 21    |
| S. Typhimurium                        | 12       | 0       | 0        | 12    |
| S. Schwarzengrund                     | 9        | 1       | 0        | 10    |
| *Salmonella* serotype 4,5,12:i:-      | 1        | 0       | 1        | 2     |
| S. Corvallis                          | 2        | 1       | 0        | 6     |
| S. Choleraesuis                       | 3        | 0       | 5        | 5     |
| S. Rissen                             | 3        | 0       | 1        | 4     |
| S. Stanley                            | 2        | 0       | 1        | 3     |
| S. Agona                              | 3        | 0       | 0        | 3     |
| S. Anatum                             | 3        | 0       | 0        | 3     |
| S. Altona                             | 0        | 2       | 0        | 2     |
| S. Lexington                          | 2        | 0       | 0        | 2     |
| S. Hadar                              | 2        | 0       | 0        | 2     |
| S. Hvittingfoss                       | 0        | 1       | 0        | 1     |
| *Salmonella* serotype 3,10:-:17       | 1        | 0       | 0        | 1     |
Table 2. Percentage of antimicrobial resistance.

| Source        | Ampicillin | Amoxicillin/ clavulanic acid | Chloramphenicol | Ciprofloxacin | Cefotaxime | Nalidixic acid | Norfloxacin | Streptomycin | Tetracycline | Sulfamethoxazole/ trimethoprim |
|---------------|------------|------------------------------|-----------------|---------------|------------|----------------|-------------|--------------|--------------|--------------------------------|
| Chicken (n = 51) | 41         | 0                            | 12              | 8             | 0          | 74             | 4           | 51           | 25           | 14                             |
| Farmer (n = 11)  | 27         | 9                            | 9               | 0             | 0          | 18             | 0           | 18           | 18           | 9                              |
| Patient (n = 19) | 63         | 0                            | 21              | 0             | 26         | 63             | 0           | 32           | 47           | 26                             |
Table 3. Percent positive amplification of resistance-related genes among *Salmonella* isolates from different origins of isolation.

| Gene                | Chickens (n = 91) | Farmers (n = 11) | Patients (n = 19) | Total (n = 121) |
|---------------------|-------------------|------------------|------------------|-----------------|
| intI1               | 64% (59/91)       | 27% (3/11)       | 11% (2/19)       | 53%             |
| 5’CS-3’CS           | 79% (72/91)       | 79% (8/11)       | 58% (11/19)      | 75%             |
| blaTEM              | 60% (55/91)       | 36% (4/11)       | 42% (8/19)       | 55%             |
| blaCTX-M            | 0% (0/91)         | 0% (0/11)        | 26% (5/19)       | 4%              |
| blashV              | 0% (0/91)         | 0% (0/11)        | 0% (0/19)        | 0%              |

*intI1*, integrase gene of class 1 integron; 5’CS-3’CS, inserted cassettes between the 5’- and 3’- conserved sequences.
Table 4. The distribution of plasmid replicon types from 3 provinces.

| Plasmid incompatibility group (Inc) | Khon Kaen (Northeast province) | Ratchaburi (Western province) | Phayao (Northern province) | Total |
|-------------------------------------|--------------------------------|--------------------------------|---------------------------|-------|
| S                                   | HS                             | PS                             | S                         | HS    | PS    | %     |
| A/C                                 | 0                              | 0                              | 1                         | 4     | 0     | 0     | 6     |
| FIB                                 | 0                              | 0                              | 0                         | 0     | 0     | 1     | 1     |
| FIC                                 | 1                              | 0                              | 0                         | 0     | 0     | 0     | 1     |
| HI1                                 | 1                              | 0                              | 1                         | 0     | 0     | 0     | 3     |
| I1                                  | 15                             | 0                              | 0                         | 3     | 0     | 0     | 21    |
| Nontypable                          | 34                             | 11                             | 1                         | 33    | 0     | 3     | 12    | 68    |

S = chicken isolates; HS = farmer isolates; PS = patient isolates.
Table 5. Relationship of *Salmonella* isolates demonstrated by various typing methods.

| PFGE patterns | Serotyping | ERIC | ST | Resistance patterns | Sample No. | Provinces |
|---------------|------------|------|----|---------------------|------------|-----------|
| P1            | S. 4, 5, 12:i:- | ERIC_Sal08 | 34 | S-TE | S42, S45 | Khon Kaen |
| P1            | S. 4, 5, 12:i:- | ERIC_Sal08 | 34 | AMP-S-TE | HS3 | Khon Kaen, |
| P2            | S. Typhimurium | ERIC_Sal08 | 213 | NA-S | S40 | Khon Kaen |
| P2            | S. Typhimurium | ERIC_Sal08 | 213 | AMP-NA-S | S47 | Khon Kaen |
| P2            | S. Typhimurium | ERIC_Sal08 | SLV-213 | NA-S | S51 | Khon Kaen |
| P3            | S. Corvallis | ERIC_Sal04 | SLV-1541 | Intermediate | HS7, HS11 | Khon Kaen |
| P3A           | S. Corvallis | ERIC_Sal04 | SLV-1541 | AMP-S | S7 | Khon Kaen |
| P3B           | S. Corvallis | ERIC_Sal04 | SLV-1541 | Intermediate | HS6 | Khon Kaen |
| P4            | S. Schwarzengrund | ERIC_Sal03 | 96 | AMP-NA | S2, HS8 | Khon Kaen |
| P5            | S. Choleraesuis | ERIC_Sal09 | 139 | AMP-C-CTX-NA-S-TE-SXT | PS17 | Ratchaburi |
| P5A           | S. Choleraesuis | ERIC_Sal09 | 139 | AMP-C-CTX-NA-S-TE-SXT | PS18 | Ratchaburi |
| P5B           | S. Choleraesuis | ERIC_Sal45 | Not done | AMP-CTX-NA-S-TE-SXT | PS19 | Ratchaburi |
| P6            | S. 4, 5, 12:i:- | ERIC_Sal08 | 34 | AMP-CTX-TE | PS8 | Phayao |
| P6            | S. 4, 5, 12:i:- | ERIC_Sal08 | 34 | AMP-S-TE | PS16 | Phayao |
| P7            | S. Rissen | ERIC_Sal02 | 469 | AMP-CTX-TE | S8 | Khon Kaen |
| P7            | S. Rissen | ERIC_Sal02 | 469 | C-TE | S14 | Khon Kaen |
| n.a.          | S. Stanley | ERIC_Sal01 | 29 | AMP | HS2 | Khon Kaen |
| n.a.          | S.     | ERIC_Sal03 | Not done | AMP-NA | S6,S12,S15 | Khon Kaen |
| Pulsotype     | Strain         | ERIC_Sal   | Resistance | Site   | Genotype |
|---------------|----------------|------------|------------|--------|----------|
| n.a.          | *S. Altona*    | Sal05      | Intermediate | HS5    | Khon Kaen |
| n.a.          | *S. Agona*     | Sal06      | S-TE       | S23    | Khon Kaen |
| n.a.          | *S. Enteritidis* | Sal07     | NA         | S34, PS1 | Phayao   |
| n.a.          | *S. Anatum*    | Sal19      | AMP-C-S-TE- | S1     | Khon Kaen |
| n.a.          | *S. Lexington* | Sal17      | Not done   | Intermediate | S4       | Khon Kaen |
| n.a.          | *S. Choleraesuis* | Sal41    | C-NA-S-TE- | PS4    | Khon Kaen |
| n.a.          | *S. Choleraesuis* | Sal42    | AMP-C-CTX- | PS6    | Khon Kaen |

Intermediate = Intermediate resistant to certain antimicrobial agents in this study; n.a., Pulsotypes were not assigned for these unique PFGE patterns. AMP = ampicillin; C = chloramphenicol; CIP = ciprofloxacin; CTX = cefotaxime; NA = nalidixic acid; NOR = norfloxacin; S = streptomycin; TE = tetracycline; SXT = sulfamethoxazole/trimethoprim
Figure 1. The distribution of MLST sequence types of *Salmonella* isolates in 4 provinces. S = chicken isolates; HS = farmer isolates; PS = patient isolates.