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Original Article

Molecular Characterization of Antibiotic-Resistant Bacteria in Contaminated Chicken Meat Sold at Supermarkets in Bangkok, Thailand

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SUMMARY: We assessed contamination by antibiotic-resistant bacteria in chicken meat obtained from supermarkets in Bangkok, Thailand. The prevalence of *Salmonella enterica* and *Escherichia coli* was 18.7% (14/75) and 53% (106/200), respectively. Most probable number (MPN) analysis showed that 56.7% of the samples (34/60) were in violation of the limit of allowable coliform bacteria in chicken meat, for which the maximum is 46,000 MPN/g. Multidrug-resistant phenotypes of both *S. enterica* and *E. coli* were found. The presence of class 1 integrons was demonstrated by polymerase chain reaction (PCR) and dot-blot hybridization. PCR showed that class 1 integrons were present in 42.9% (6/14) and 37.7% (40/106) of *S. enterica* and *E. coli* isolates, respectively. Resistance genes identified in this study were *aadA2*, *aadA4*, *aadA22*, and *aadA23* (for aminoglycoside resistance); *dfrA5* (for trimethoprim resistance), and *lnuF* (for lincosamide resistance). Four *S. enterica* isolates underwent multilocus sequence typing and the results were sequence type (ST) 50, ST 96, ST 1543, and ST 1549, which matched well with strains from many countries and reflected an international spread. Our study revealed that class 1 integrons have spread into community sources and might play an important role in horizontal antibiotic resistance gene transfer.

INTRODUCTION

An increase in the prevalence of antibiotic-resistant bacteria is becoming a major public health concern worldwide because of its effects on treatment outcomes and medical costs. Many factors are involved in the emergence of resistant strains, particularly the misuse of antibiotics in both humans and animals (1). The correlation between antibiotics misuse in animals and the emergence of antibiotic-resistant bacteria has been well documented (2–4). Food-producing animals, including swine, poultry, and cattle, account for reservoirs of antibiotic-resistant bacteria, especially *Salmonella enterica* and *Escherichia coli*, and play an important role in the spread of antibiotic-resistant bacteria via undercooked foods. The contamination of food-producing animals with antibiotic-resistant *S. enterica* and *E. coli* has been reported worldwide (5–10). Another concern is multidrug-resistant (MDR) bacteria, which have exhibited resistance to a variety of antibiotics, and their potential spread.

MDR bacteria are able to counteract antibiotics treatment by acquiring resistance genes, spontaneous mutation, and disseminating resistance genes via mobile genetic elements (i.e., plasmid, transposon, and insertion sequences) (11). Subsequently, these bacteria develop a unique element known as an integron element to increase the efficiency with which they capture and spread their resistance determinants to other bacteria (12). Integrons are defined as an assembly platform that incorporates open reading frames of resistance genes in the form of gene cassettes that integrate by site-specific recombination mediated by an integrase (13,14). The principle of integrons comprises 3 key elements necessary for capturing resistance genes: integrase encoded by *intI*, which belongs to the tyrosine recombinase family; a primary recombination site called *attI*; and a strong promoter called P_c (14). The 5 integron classes are based on integrase sequences that exhibit 40–58% identity (14,15), and class 1 integrons have been detected extensively in clinically important bacteria. Although integrons are not classified as a mobile genetic element, they have been shown to be associated with other mobile genetic elements (e.g., plasmids) and lead to rapid spread among pathogenic and commensal bacteria (16). Although commensal bacteria are not responsible for causing diseases directly, their role in the reservoir of resistance genes is a concern. Contamination by class 1 integrons bearing *S. enterica* and *E. coli* in food-producing animals has been demonstrated worldwide (6,17,18). Additionally, clonal spread plays an important role and is also responsible for the spread of antibiotic-resistant bacteria. A well-known example is *Salmonella* Typhimurium, definitive phage type 104 (DT 104), which emerged in cattle in the 1990s and spread globally (19).

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In Thailand, there have been many studies on the prevalence of antibiotic-resistant bacteria in food-producing animals (8,10,20), but little is known about the genetic mechanism conferring antibiotic resistance and its molecular characteristics. This study therefore aimed to determine the prevalence, molecular characteristics, and epidemiology of antibiotic-resistant *S. enterica* and *E. coli* from fresh chicken meat sold at supermarkets in Bangkok, Thailand.

MATERIALS AND METHODS

Sample collection: In total, 200 pre-packaged raw chicken meat samples, which were distributed by 4 major companies, were obtained randomly from 7 supermarkets in Bangkok from July 2010 to May 2011. All samples were transported on ice and processed for microbiological tests using aseptic technique within 30 min after purchase.

Bacterial isolation: *Salmonella* was isolated according to a standard method described in ISO 6579 (21), and an appropriate method was used to isolate non-pathogenic *E. coli*. Twenty-five grams of chicken meat was weighed and aseptically placed in a sterile stomacher bag with 225 ml buffered peptone water. The contents of the stomacher bag were then homogenized and incubated for 18 h at 37°C.

For isolation of *Salmonella*, 1 ml and 0.1 ml chicken meat rinse solutions were transferred to 2 tubes containing 10 ml tetrathionate broth (TT broth) and Rappaport-Vassiliadis broth (RV broth), respectively, and incubated at 37°C for 24 h and 42°C for 24 h, respectively. The broth cultures were then streaked on Salmonella-Shigella agar and deoxycholate-hydrogen sulfide-lactose agar and incubated at 37°C for 24 h. Typical *Salmonella* colonies were confirmed using a biochemical identification test. All *Salmonella* were typed further into serotypes.

For *E. coli* isolation, chicken meat rinse solution was streaked on MacConkey agar and incubated at 37°C for 24 h. Typical *E. coli* colonies were confirmed with a biochemical identification test.

***Salmonella* serotyping:** All isolates were serotyped by using slide agglutination for O antigens (22) and the Gard technique for H antigens (23) with hyperimmune sera (S & A Reagents Lab, Bangkok, Thailand) and interpreted according to the Kauffmann-White scheme (24).

Most probable number (MPN): MPN analysis was performed to determine the total presence of coliform bacteria in raw chicken meat by using the 3 tubes protocol (25). Briefly, 11 g chicken meat was mixed in 99 ml buffered peptone water and further diluted to 10^{-2}, 10^{-3}, and 10^{-4} dilution before inoculation into lauryl sulphate tryptose broth. The total number of positive tubes at each dilution was determined and used to interpret the MPN value. The presumptive-positive lauryl sulphate tryptose broth was confirmed by inoculating the contents into brilliant green lactose bile broth, and the result was interpreted according to the number of brilliant green lactose bile positive tubes.

Antimicrobial susceptibility testing (AST): AST of *S. enterica* and *E. coli* was performed using the disk diffusion method according to the standard procedure of the Clinical and Laboratory Standards Institute (CLSI) (26). The antibiotics used in this study were ampicillin (10 μg), amoxicillin-clavulanic acid (20/10 μg), cephalothin (30 μg), cefuroxime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefepime (30 μg), ertapenem (10 μg), imipenem (10 μg), meropenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), chloramphenicol (30 μg), and tetracycline (30 μg). *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *E. coli* ATCC 35218 were used as quality control strains.

Detection of class 1 integrons: Polymerase chain reactions (PCR) with specific primers for the *intI1* gene and an internal segment between 5′ CS and 3′ CS were performed. The *intI1*-specific primers were *intI1*-F (5′-AAGGATCGGGCCTTGATGTT-3′) and *intI1*-R (5′-CAGCGCATCAAGCGGTGAGC-3′), which produce a 471-bp PCR product. The PCR for *intI1* was as follows: pre-denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 7 min. The internal segments between 5′ CS and 3′ CS were detected using primers as described previously (27). The PCR was carried out with pre-denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 7 min. Resistance gene cassettes inserted in the integron were confirmed by DNA sequencing. PA 67, a *Pseudomonas aeruginosa* clinical isolate from Thailand containing 1,897 bp integron cassettes (accession no. AY553333) was used as a positive control in both tests.

Plasmid profile study: A plasmid profile was used to determine genetic relatedness among integrons carrying *S. enterica* and *E. coli* by using the alkaline lysis method, followed by restriction enzyme analysis.

Randomly amplified polymorphic DNA (RAPD) PCR: RAPD-PCR was performed using an R018 primer (5′-GTA TTG CCC T-3′). PCR was performed under the following conditions: pre-denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 42°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 7 min.

Dot-blot hybridization: A PCR product from the *intI1* primer was used to construct the *intI1*-specific hybridization probe. The *intI1* probe was labeled using a High Prime DNA Labeling Kit (Roche Applied Science, Mannheim, Germany). Detection was performed by using a CDP-Star detection module (Amersham Pharmacia Biotech, Piscataway, N.J., USA).

Multilocus sequence typing (MLST): Four *S. enterica* isolates were selected for MLST analysis. Primer sequences and PCR conditions are described in the UCC *Salmonella enterica* MLST database (http://mlst.ucc.ie/mlst/dbs/Senterica).

RESULTS

Prevalence and MPN analysis: *S. enterica* was isolated in 14 (18.7%) of 75 samples. Serogroup B was the most common serogroup (6 isolates) in this study, followed by serogroup C3 (4 isolates), and serotypes

Table 1. Result of *intI1* PCR and resistance rate of *S. enterica* and *E. coli*

	intI1 PCR (%)	Percentage of resistance (%)																	
		AM	AMC	CF	CXM	CTX	CRO	CAZ	FEP	IPM	MEM	ETP	CIP	LEV	GM	AK	SXT	TE	C
S. enterica	42.9	78.6	0	0	0	0	0	0	0	0	0	0	0	0	71.4	0	17	21.4	ND
E. coli	37.7	72.3	6.4	27.7	2.1	0	0	0	0	0	0	0	10.6	10.6	38.3	0	7.1	48.5	17.2

A total of 14 isolates and 46 isolates of *S. enterica* and *E. coli* were used to calculate the resistance rate.
AM, ampicillin; AMC, amoxicillin-clavulanic acid; CF, cephalothin; CXM, cefuroxime; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; ETP, ertapenem; CIP, ciprofloxacin; LEV, levofloxacin; GM, gentamicin; AK, amikacin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline; C, chloramphenicol; ND, not done.

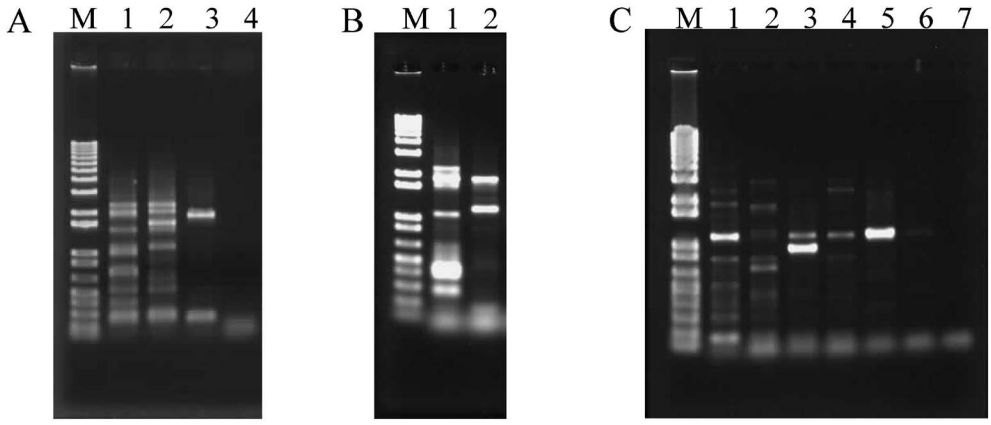

Fig. 1. (A) PCR by using 5′ CS and 3′ CS primers of 2 *E. coli* isolates. Lane M, 1 kb plus DNA ladder; lane 1, E9; lane 2, E12; lane 3, positive control; lane 4, negative control. (B) PCR by using 5′ CS and 3′ CS primers of 2 integrase-negative isolates. Lane M, 1 kb plus DNA ladder; lane 1, *E. coli* E17; lane 2, *S.* Kedougou Sal130. (C) PCR by using 5′ CS and 3′ CS primers of 6 integron-carrying *S. enterica* isolates. Lane M, 1 kb plus DNA ladder; lane 1, Sal143; lane 2, Sal145; lane 3, Sal162; lane 4, Sal165; lane 5, Sal178; lane 6, Sal180; lane 7, negative control.

Kedougou, Saintpaul, Schwarzengrund, Agona, Orion, Manhattan, Albany, Kiambu, Altona, and 4,12,27:d:- were identified (see Table 4). *E. coli* was detected in 106 (53%) of 200 samples. We used the MPN technique to determine the total coliform presence in chicken meat, and the percentage of unacceptable quality in the meat was high (56.7%, 34/60); according to the criteria of the Department of Medical Sciences, Ministry of Public Health, the quality must be <500 MPN/g.

Antibiotic resistance profile: Most *S. enterica* isolates exhibited resistance to ampicillin (78.6%), tetracycline (21.4%), and gentamicin (71.4%). In *E. coli* isolates, we observed resistance to ampicillin (72.3%), tetracycline (48.5%), gentamicin (38.3%), and cephalothin (27.7%) (Table 1). However, resistance to fluoroquinolones, chloramphenicol, trimethoprim-sulfamethoxazole, and amoxicillin-clavulanic acid was also observed in some isolates. The ampicillin- and gentamicin-resistant phenotype was detected in 14 isolates (8 *S. enterica* and 6 *E. coli*). Extended spectrum β-lactamases-producing isolates, as screened by the disk diffusion method according to CLSI 2010, were not detected in the *S. enterica* or *E. coli* isolates. Nevertheless, MDR phenotype (resistance to at least 3 classes of antibiotics) *S. enterica* and *E. coli* isolates were found (Table 2).

Prevalence of class 1 integrons and identification of resistance cassettes: All *S. enterica* and *E. coli* isolates were screened for the presence of class 1 integrons by *intI1*-specific PCR. Integron-harboring *S. enterica* and *E. coli* were detected at rates of 42.9% (6/14) and

Table 2. Resistance phenotypes of *S. enterica* and *E. coli*

Resistance phenotype	*S. enterica* (no. of isolates)	*E. coli* (no. of isolates)
AM	0	3
AMC	0	1
CF	0	1
TE	0	1
AM, CF	0	4
AMC, CF	0	1
AM, GM	8	6
AM, TE	0	1
CF, CXM	0	1
AM, AMC, CF	0	1
AM, CF, GM	0	1
AM, CIP, LEV	0	1
AM, GM, TE	2	5
AM, SXT, TE	1	2
AM, CF, TE	0	1
AM, CIP, LEV, GM	0	2
AM, GM, TE, C	0	1
AM, CIP, LEV, TE	0	1
AM, CF, SXT, C	0	1
AM, SXT, TE, C	0	2
CIP, LEV, GM, SXT	0	1
AM, CF, GM, SXT, TE	0	1
AM, CF, GM, SXT, TE, C	0	1
Susceptible strain	3	6

Abbreviations are in Table 1.

Table 3. Features of *S. enterica* and *E. coli* carrying class 1 integrons in this study

	PCR for *intI1*	Dot blot hybridization	5′ CS–3′ CS	Resistance cassette	Resistance phenotype
E9 (*E. coli*)	+	+	1 kb	*aadA23*	AM, CIP, LEV, GM
E9 (*E. coli*)	+	+	700 bp	*dfrA5*	AM, CIP, LEV, GM
E12 (*E. coli*)	+	+	850 bp	*aadA4*	CIP, LEV, GM, SXT
E17 (*E. coli*)	−	+	500 bp	*lnuF*	AM, CF, GM
Sal130 (*S.* Kedougou)	−	−	1.2 kb	*aadA2, lnuF*	AM, GM
Sal162 (*S.* Kedougou)	+	−	1 kb	*aadA22*	AM, GM, TE

Abbreviations are in Table 1.

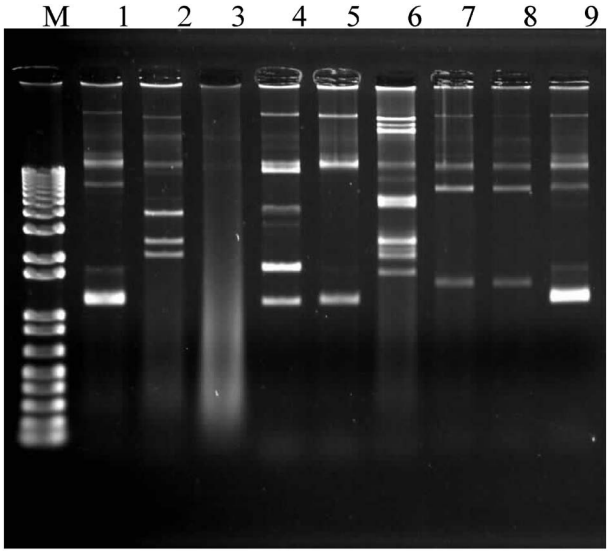

Fig. 2. Plasmid profiles of *E. coli* carrying integron isolated from chicken meats. Lane M, 1 kb plus DNA ladder; lane 1, E60; lane 2, E123; lane 3, E134; lane 4, E166; lane 5, E169; lane 6, E173; lane 7, E181; lane 8, E183; lane 9, E186.

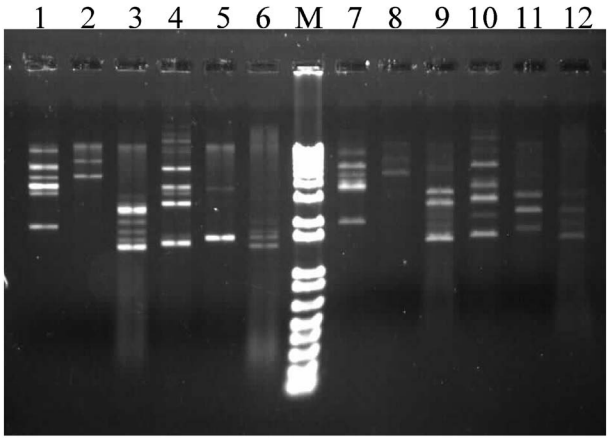

Fig. 3. Plasmid profiles of 6 *S. enterica* carrying integron isolates and their *Eco*RI restriction enzyme analysis. Lane M, 1 kb plus DNA ladder; lane 1, Sal143; lane 2, Sal145; lane 3, Sal162; lane 4, Sal165; lane 5, Sal178; lane 6, Sal 180; lane 7–12, *Eco*RI digested products plasmid of Sal143, Sal145, Sal162, Sal165, Sal178, and Sal180, respectively.

37.7% (40/106), respectively. Resistance cassettes in integron carrying *S. enterica* and *E. coli* isolates (PCR-positive for *intI1*) were identified by using 5′CS and 3′CS-specific PCR. Multiple bands were obtained (Fig.

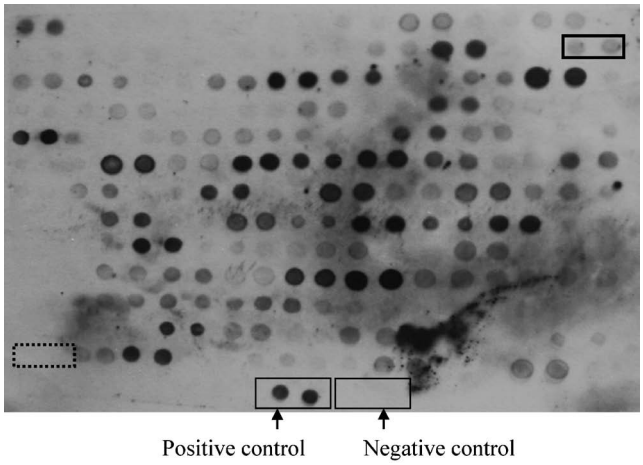

Fig. 4. Dot blot hybridization of *S. enterica* and *E. coli* by using *intI1* specific probe. Solid-lined box represents E17 and dashed box represents Sal130. Positive control and negative control are shown as indicated by arrow heads.

1A–1C). We selected *E. coli* isolates (E9 and E12) that exhibited resistance to various classes of antibiotics (E9 was resistant to β-lactam, fluoroquinolones, and aminoglycosides, and E12 was resistant to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole), and *S.* Kedougou (Sal162) to perform DNA sequencing by cloning PCR fragments with a StrataClone™ PCR Cloning Kit. The resistance cassettes were found not only in the integrase-positive isolates, but also in some integrase-negative isolates (E17 and Sal130), as demonstrated by the PCR results (Fig. 1B). The 5′ CS and 3′ CS PCR result of 6 integron-carrying *S. enterica* isolates is shown in Fig. 1C. The resistance genes found in this study were *aadA2*, *aadA4*, *aadA22*, and *aadA23* (for streptomycin and spectinomycin resistance), *dfrA5* (for trimethoprim resistance), and *lnuF* (for lincosamide resistance) (Table 3).

Plasmid profile: The plasmid profile was used to determine the genetic relatedness among integron-carrying isolates. We identified 25 plasmid patterns from 33 integron-carrying *E. coli* isolates. Nevertheless, the same plasmid patterns were found in *E. coli* isolates obtained from identical (E181 and E183) and different locations (E60 and E186) (Fig. 2). A unique plasmid pattern was demonstrated among 6 integron-carrying *S. enterica* isolates (Fig. 3).

Dot-blot hybridization: The results of resistance cassettes identified in integrase-negative isolates (E17 and

Sal130) indicated either low PCR detection sensitivity or a diverse *intI1* sequence. Therefore, dot-blot hybridization with an *intI1*-specific probe was used to confirm the PCR results. Dot-blot hybridization could detect the E17 isolate, but not the Sal130 isolate (Fig. 4). There was equality of detection between PCR and dot-blot hybridization for *S. enterica*, where 6 of 14 *S. enterica* isolates (42.9%) were detected (Fig. 5). Dot-blot hybridization could also detect 61 of 106 *E. coli* isolates (57.6%), and yielded a higher rate of detection when compared with PCR (37.7%).

RAPD-PCR of *Salmonella*: A total of 9 RAPD patterns were detected among 14 *S. enterica* isolates (Fig. 6). Identical RAPD patterns (patterns I, II, III, and IX) among *S. enterica* isolates demonstrated the clonal relatedness of these isolates. Therefore, we used various typing methods (i.e., serogrouping, serotyping, an-

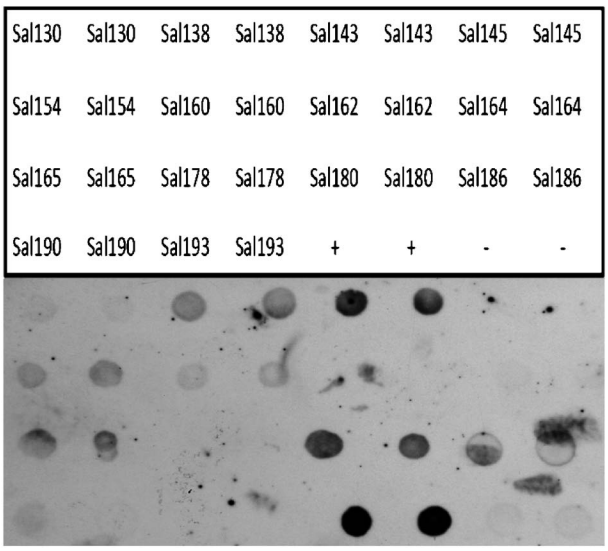

Fig. 5. Dot blot hybridization in duplicates of 14 *S. enterica* isolates by using *intI1* specific probe.

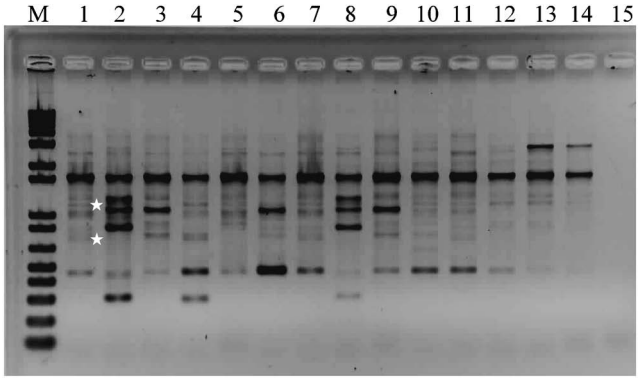

Fig. 6. RAPD-PCR of *Salmonella* isolates. Lane M, 1 kb plus DNA ladder; lane 1, Sal130; lane 2, Sal138; lane 3, Sal143; lane 4, Sal145; lane 5, Sal154; lane 6, Sal160; lane 7, Sal162; lane 8, Sal164; lane 9, Sal165; lane 10, Sal178; lane 11, Sal180; lane 12, Sal186; lane 13, Sal190; lane 14, Sal193; lane 15, negative control. White stars indicate the different banding positions between pattern I (lane 1) and pattern V (lane 5). (Photo is in negative color.)

Table 4. Relationships of *Salmonella* isolates demonstrated by various typing methods

Isolate	PCR for *intI1*	Dot blot detection of *intI1*	Serogrouping	Serotyping	RAPD-PCR	Resistance phenotype	Place of collection[1)]	Collection date
Sal130	−	−	G2	Kedougou	I	AM, GM	A1	Jan 2, 2011
Sal138	−	+	B	Saintpaul	II	AM, GM	B2	Mar 14, 2011
Sal143	+	+	B	Schwarzengrund	III	AM, GM	B2	Mar 14, 2011
Sal145	+	−	B	Agona	IV	—	B2	Mar 14, 2011
Sal154	−	+	E	Orion	V	AM, GM	A3	Apr 4, 2011
Sal160	−	+	C2	Manhattan	VI	AM, GM	A3	Apr 4, 2011
Sal162	+	−	G2	Kedougou	I	AM, GM, TE	A1	Apr 4, 2011
Sal164	−	−	B	Saintpaul	II	AM, GM	A1	Apr 4, 2011
Sal165	+	+	B	4,12,27:d:-	III	AM, GM	A1	Apr 4, 2011
Sal178	+	−	C3	Albany	VII	AM, SXT, TE	A1	Apr 19, 2011
Sal180	+	+	C3	Albany	I	AM, GM, TE	A1	Apr 19, 2011
Sal186	−	−	B	Kiambu	VIII	—	C4	Apr 25, 2011
Sal190	−	−	C3	Altona	IX	—	C4	Apr 25, 2011
Sal193	−	−	C3	Altona	IX	AM, GM	A3	May 3, 2011

[1)]: A, B, and C represent supermarkets, whereas 1, 2, 3, and 4 represent major distributor companies.
Shaded rows indicate selected isolates for MLST typing.
Abbreviations are in Table 1.

Table 5. MLST allelic profiles of four *S. enterica* isolates in this study

Sample	Allelic profile *aroC-dnaN-hemD-hisD-purE-sucA-thrA*	ST	Country
Sal138	5-21-18-9-6-12-17	50	Chile, Denmark, U.S.A., Germany, Australia
Sal162	417-4-15-262-95-9-141	1543	Vietnam
Sal165	43-47-49-49-41-15-3	96	Tunisia, U.S.A., Taiwan, Denmark, Scotland
Sal193	347-394-78-496-426-9-102	1549	Vietnam

tibiotic resistance phenotype) and RAPD-PCR, incorporated with the collection data to characterize the *S. enterica* isolates. Four repetitive RAPD patterns (patterns I, II, III, and IX) exhibited good correlation with the serogrouping and serotyping results (Table 4). Moreover, these well-correlated strains were obtained at different times.

MLST of *S. enterica*: Four *S. enterica* isolates (Sal138, Sal162, Sal165, and Sal193), which presented RAPD patterns II, I, III, and IX, respectively, were subjected to MLST analysis. The MLST revealed 4 sequence types (ST): ST 50, ST 96, ST 1543, and ST 1549 (Table 5).

DISCUSSION

The results demonstrated that the chicken meat obtained from supermarkets in Bangkok, Thailand, was heavily contaminated with enteric bacteria. Unfortunately, TT broth, the cheaper and more convenient enrichment media for *S. enterica* that we had modified from RV broth according to the standard procedure described for *S. enterica* isolation, ISO 6579, could not detect any of 125 chicken samples. RV broth has been demonstrated to identify more *Salmonella*-positive samples than enrichment in TT broth (28), and is suitable for high microbial-load foods (29) as opposed to TT broth, which is suitable for low microbial-load foods (30). In addition, TT broth incubated at 43°C has been used for high microbial-load foods, in contrast to the incubation at 35°C in our study (29). The MPN analysis reflected a high microbial load in our chicken meat samples, 56.7% of which had an MPN value greater than 500 per gram of chicken meat, the maximum value of which is 46,000 MPN/g. In this study, the MPN results determined that the chicken meat was a high microbial-load food that preferred TT broth incubated at 43°C or RV broth in the enrichment step. The prevalence of *S. enterica* (18.7%) was higher (9,31) and lower (32–34) than that reported from other countries. In Thailand, a study reported a higher prevalence than that in this study (10). The serotypes present in this study were diverse, of which *S.* Kedougou, *S.* Albany, and *S.* Altona were the most common. The serotype prevalence in this study correlated with the top of *Salmonella* serotypes in animals in Thailand (35–37). The other serotypes found in this study (i.e., *S.* Agona and *S.* Schwarzengrund) were also often isolated from animals. The fact that *Salmonella* serotypes in animals were similar to those in humans (35–37) indicates the role of contaminated foods in the spread of *Salmonella* to humans. Interestingly, *S.* Schwarzengrund, which has been reported to be an internationally spread strain (38), was found in this study. A high percentage of *E. coli* contamination was also observed.

Most *S. enterica* and *E. coli* isolates were resistant to at least one class of antibiotics, and both exhibited a high rate of resistance to ampicillin, gentamicin, and tetracycline (Table 1). There were high rates of ampicillin and gentamicin resistance in both *S. enterica* and *E. coli*, which suggested high use of ampicillin and gentamicin in farming. *E. coli* was more resistant than *Salmonella*, and may be a good reservoir of resistance genes for *Salmonella* (Tables 1 and 2). The use of antibiotics in animals in Thailand could not be demonstrated clearly; however, the resistance pattern found in this study is likely the result of such an event, as supported by previous studies (2–4). Class 1 integrons, an efficient tool for the capture and spread of antibiotic resistance genes (14), were found in *S. enterica* and *E. coli*. The finding that 42.9% and 37.7% of *S. enterica* and *E. coli* isolates, respectively, carried class 1 integrons emphasizes an important role for class 1 integrons in conferring resistant phenotypes among these bacteria. Various gene cassettes were identified (i.e., *aadA2*, *aadA4*, *aadA22*, *aadA23*, *dfrA5*, and *lnuF*). Most characterized isolates contained cassettes for aminoglycoside resistance, and these cassettes are common in class 1 integrons (39–41). Interestingly, *lnuF*, which confers lincosamide resistance and is present in class 1 integrons, was rarely detected in Gram-negative bacteria, which indicated the occurrence of resistance gene transfer from Gram-positive bacteria to Gram-negative bacteria or vice versa. *lnuF* was reported in class 2 integrons by Ruiz et al. (41). Additionally, we found that integrase-negative bacteria also carried resistance gene cassettes (Table 3). The reasonable explanation is the low sensitivity of the PCR method, which was due to mismatch of the annealing position of the primers in comparison with homologous hybridization of the probe in dot-blot hybridization or sequence diversity in *intI1*. Dot-blot hybridization underscored the low sensitivity of the PCR method and had a higher rate of detection for *E. coli*. The failure of *intI1* detection by PCR and dot-blot hybridization in the integrase-negative isolate Sal130 may indicate the possible deletion of *intI1*. Although dot-blot hybridization could restore the PCR result for E17, it produced the same result as PCR for Sal130 (Fig. 4).

We assessed the genetic relatedness among these bacteria by using typing methods based on phenotypic and genotypic backgrounds. The same plasmid patterns found in *E. coli* indicate that clonal expansion also plays a role in bacterial spread. The *Salmonella* typing scheme was divided into the phenotypic method, comprising serotyping and resistance patterns incorporated with demographic data, and the genotypic method, comprising RAPD-PCR, plasmid profile study, and MLST. The result of various typing methods incorporated with demographic data might support the role of clonal spread among these bacteria (Table 4). Nevertheless, these typing methods have some limitations (e.g., reproducibility), and certain discrepancies of serotypes and other evolutional genetic markers may arise from horizontal gene transfer (42). Inter-laboratory comparison is another limitation intrinsic to typing methods based on their continuous data values (e.g., difficulty of RAPD-PCR discrimination between patterns I and V) (Fig. 6). MLST is a portable, accurate, and highly discriminating typing system. Through data base analysis, the STs of our strains were matched in strains isolated in other countries (Table 5). MLST analysis combined with serotyping could be helpful for further characterization of obtained *Salmonella* strains (43). The recently published review on new alternative methodologies for *S. enterica* subsp. *enterica* subtyping by Wattiau et al. emphasizes the importance of DNA-based typing schemes (44). Furthermore, the latest MLST findings

can demonstrate evolutional grouping better than serotyping, which sometimes can mislead an epidemiological investigation (45).

In conclusion, we demonstrated that antibiotic-resistant bacteria and integron elements have been spread among food-producing animals and can be spread to humans through consumption of undercooked food. Antibiotic use in humans and animals should be more closely monitored to limit the emergence of antibiotic-resistant bacteria. The MLST data can be recorded and compared in future epidemiological analyses.

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Conflict of interest None to declare.

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