FULL PAPER *Public Health*

Prevalence and Characteristics of *Salmonella* spp. Isolated from Poultry Slaughterhouses in Korea

Dong Hwa BAE[1)], Hirut Kidie DESSIE[1)], Ho Jeong BAEK[1)], Seong Guk KIM[2)], Hee Soo LEE[3)] and Young Ju LEE[1)]*

[1)]*College of Veterinary Medicine, Kyungpook National University, Daegu 702–701, Republic of Korea*
[2)]*Gyeongbuk Veterinary Service Laboratory, Daegu 702–210, Republic of Korea*
[3)]*Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang 430–757, Republic of Korea*

(Received 20 February 2013/Accepted 29 April 2013/Published online in J-STAGE 13 May 2013)

ABSTRACT. Poultry products have consistently been identified as important sources of *Salmonella* infection in humans, because vertical transfer of infection from breeding hens to progeny is an important aspect of the epidemiology of *Salmonella* spp. infection within the poultry industry. The aim of this study was to estimate the prevalence of *Salmonella* contamination in poultry products from 15 different located geographical areas from among the 50 poultry slaughterhouses authorized to operate in Korea and to characterize all the isolates by genotyping, phage typing and antibiotic resistance pattern. *Salmonella* was isolated from 10 (66.7%) of the first and 5 (33.3%) of the last chilling waters and from 32 (42.7%) carcasses originating from 9 slaughterhouses. The major prevalent serotypes of *Salmonella* originating from 2 duck slaughterhouses and 13 chicken slaughterhouses tested were *S.* Typhimurium and *S.* Enteritidis, respectively. Regarding the characteristics of their antibiotic resistance, 8 of the 11 ampicillin resistant (Am^R) isolates carried bla_{TEM} only, two carried bla_{TEM} and $bla_{CTX\text{-}M\text{-}14}$ and one carried $bla_{CTX\text{-}M\text{-}3}$ and only one Am^R isolate with the $bla_{CTX\text{-}M\text{-}3}$ β-lactamase gene was an ESBL-producing *Salmonella* strain. Twenty-seven *Salmonella* isolates showed nalidixic acid resistance with a mutation at amino acid codon Asp87 in *gyrA* and no mutation in the *parC* gene. In all the phenotypic and genotypic properties of the 18 *S.* Enteritidis and 8 *S.* Typhimurium based on PFGE, phage types and antibiotic resistance pattern, the predominant patterns were XEI/BEI-PT32a-Na^R (n=5) and XTI/BTI-RNDC-no resistant antibiotics (n=6), respectively.

KEY WORDS: β-lactamase, *gyrA*, poultry slaughterhouse, prevalence, *Salmonella*.

doi: 10.1292/jvms.13-0093; *J. Vet. Med. Sci.* 75(9): 1193–1200, 2013

Salmonellosis is one of the serious diseases responsible for numerous cases of foodborne illnesses in the world. A great increase in human food-borne infections caused by *Salmonella* including *Salmonella enteritidis* (*S.* Enteritidis) and *Salmonella typhimurium* (*S.* Typhimurium) has been noted in the United States, Europe and Korea [5, 21, 23]. *Salmonella* infections in humans often result from the ingestion of contaminated foods, such as poultry, beef, pork, eggs and produce. Estimates from the Centers for Disease Control and Prevention (CDC) reported that more than a million people have *Salmonella* poisoning every year from a variety of causes. About 25,000 people get so sick they seek treatment at a hospital, and about 500 people die every year. The Korea CDC also reported that the prevalence of *Salmonella* spp. in food, especially in poultry products (up to 2.2%), is high in Korea [12] and was responsible for over 63% of food-borne illnesses recorded from 2004 to 2005 [26].

Poultry products have consistently been identified as important sources of *Salmonella* infection in humans, because vertical transfer of infection from breeding hens to progeny is an important aspect of the epidemiology of *Salmonella* spp. infection within the poultry industry [17]. In one of our earlier studies, we showed that a great number of *Salmonella* spp. contaminate poultry products and have somewhat different genetic types according to the origin of the integrated broiler operation [25]. Nevertheless, there have been no studies following the dissemination of *Salmonella* in the entire poultry industry in Korea.

The aim of this study was to estimate the prevalence *Salmonella* contamination in poultry products from 15 different located geographical areas from among the 50 poultry slaughterhouses authorized in Korea and to characterize all the isolates by genotyping, phage typing and antibiotic resistance pattern.

MATERIALS AND METHODS

Sample collection: Samples were obtained from 2 of the 9 duck slaughterhouses and from 13 of the 41 chicken slaughterhouses authorized in Korea, respectively. The first chilling water, the last chilling water and 5 carcasses from each slaughterhouse were sampled. The water samples from the first and last chiller tanks were collected during the processing of carcasses and transferred to sterile polyethylene bags (500 m*l*). Carcasses were collected from the rehang belt prior to the rehanging of the carcasses on the drip line. Each carcass was aseptically placed into a vacuum bag (Sealed Air, Elmwood Park, NJ, U.S.A.), and 400 m*l* of sterile buffered peptone water (BPW; Difco, Sparks, MD, U.S.A.) was added to the bag. The bag was shaken 50 times, and approximately 50 m*l* of rinse water was transferred into a

*CORRESPONDENCE TO: LEE, Y.J., College of Veterinary Medicine, Kyungpook National University, Daegu 702–701, Republic of Korea.
e-mail: youngju@knu.ac.kr

sterile specimen cup.

Bacterial isolation: Approximately 25 m*l* of chilling water or carcass rinse fluid was added to 225 m*l* BPW and incubated at 35 ± 2°C for 20–24 hr. After pre-enrichment, 0.1 m*l* of the broth was transferred into a 10 m*l* Rappaport-Vassiliadis broth (RV broth; Difco), which was prepared according to the manufacturer's instructions. The RV broth was incubated overnight at 41.5°C and streaked onto Rambach agar (Difco). Two typical colonies picked from the samples were serotyped by slide and tube agglutination using O and H antiserum (Difco) according to the Kauffmann and White scheme [36]. If 2 colonies showed the same serotypes and antibiotic resistant pattern, only one colony was randomly chosen and included in this study.

Antibiotic susceptibility test: All *Salmonella* isolates tested were investigated for their antibiotic resistance with the disc diffusion test using the following discs (Difco): amikacin (An, 30 μg), gentamicin (Gm, 10 μg), kanamycin (K, 30 μg), ampicillin (Am, 10 μg), cefazolin (Cz, 30 μg), cephalothin (Cf, 30 μg), cefepime (Fep, 30 μg), cefotaxime (Ctx, 30 μg), cefoxitin (Fox, 30 μg), nalidixic acid (Na, 30 μg), ciprofloxacin (Cip, 5 μg), norfloxacin (Nor, 10 μg), tetracycline (Te, 30 μg), trimethoprim/sulfamethoxazole (Sxt, 1.25/23.75 μg), chloramphenicol (C, 30 μg), imipenem (Imp, 10 μg), streptomycin (S, 10 μg) and ceftazidime (Caz, 30 μg). The results were evaluated according to the standard, M2-A9 of the Clinical and Laboratory Standards Institute [13]. For phenotypic detection of ESBL-producing isolates, they were screened using the double-disc synergy test (DDST) with aztreonam (Atm, 30 μg), Ctx, Fep, Caz and amoxicillin/clavulanic acid (Amc, 20 /10 μg) as previously described [3].

Minimal inhibition concentrations (MICs) assay: MICs were determined for the following antibiotics: Am, Ctx and Caz for phenotypic ESBL-producing isolates and Na and Cip for isolates showing Na resistance. The MICs were determined with an agar dilution method according to the standard, M7-A7 of the Clinical and Laboratory Standards Institute [13].

PCR detection of the β-lactamase genes: The presence of genes encoding TEM (forward, 5′-TTCTTGAAGACGAAAGGGC-3′; reverse, 5′-ACGCTCAGTGGAACGAAAAC-3′), SHV (forward, 5′-CACTCAAGGATGTATTGTG-3′; reverse, 5′-TTAGCGTTGCCAGTGCTCG-3′), CTX-M-3 group (forward, 5′-AATCACTGCGCCAGTTCACGCT-3′; reverse, 5′-GAACGTTTCGTCTCCCAGCTGT-3′) and CTX-M-14 group (forward, 5′-TACCGCAGATAATACGCAGGTG-3′; reverse, 5′-CAGCGTAGGTTCAGTGCGATCC-3′) β-lactamases was analyzed by PCR and sequencing as previously described [10, 35, 39].

PCR detection of the gyrA and parC genes: Fragments of the *gyrA* (forward, 5′-TGTCCGAGATGGCCTGAAGC-3′; reverse, 5′-TACCGTCATAGTTATCCACG-3′) and *parC* (forward, 5′-CTATGCGATGTCAGAGCTGG-3′; reverse, 5′-TAACAGCAGCTCGGCGTATT-3′) genes including the quinolone resistance-determining region (QRDR) responsible for quinolone resistance were amplified by PCR and sequenced as previously described [15, 18].

Phage typing: All *S.* Enteritidis and *S.* Typhimurium isolates were phage-typed at the Animal, Plant and Fisheries Quarantine and Inspection Agency (Anyang, Republic of Korea). Standard phages were obtained from the Laboratory of Enteric Pathogens, Public Health Laboratory Service (PHLS) in England. The phage lysis pattern of each culture was compared with the published patterns. Strains showing a pattern that did not conform to any recognized phage type were designated as "reaction-dose-not-conform" (RDNC).

Pulsed field gel electrophoresis (PFGE): PFGE was performed according to the "One-Day (24–28 hr) standardized Laboratory Protocol for Molecular Subtyping of Non-typhoidal *Salmonella* by PFGE" [40]. Chromosomal DNA was digested with 50 U of *Xba*I (Promega, Madison, WI, U.S.A.) or *Bln*I (Promega). PFGE was done on a CHEF Mapper XA system (Bio-Rad Lab., Richmond, CA, U.S.A.) in 0.5X Tris-Borate-EDTA buffer (Bio-Rad Lab.) with recirculation at 14°C. Pulse times were ramped from 2.2 to 63.8 sec during an 18 hr run at 6.0 V/cm. After electrophoresis, the gels were stained in 2 μg of aqueous ethidium bromide (Sigma-Aldrich. St. Louis, MO, U.S.A.) per m*l* for 15 min and were photographed using 300 nm UV light. The similarity of the PFGE patterns was calculated by means of computer-based similarity and clustering programs (BioNumerics 3.0, Applied Maths, Biosistematica, Devon, UK). Dice coefficient was used for similarity calculation, and the similarity matrix was expressed graphically by an unweighted average linkage (UPGMA). The relatedness of the PFGE profiles of *Salmonella* isolates was estimated based on the presence or absence of the shared bands.

RESULTS

The prevalence of *Salmonella* in the first and last chilling waters and the 5 carcasses sampled from each of the 15 poultry slaughterhouses is presented in Table 1. *Salmonella* was isolated from 10 (66.7%) of the first chilling waters, 5 (33.3%) of the last chilling waters and from 32 (42.7%) carcasses originating from 9 slaughterhouses. All of the samples from 2 duck slaughterhouses were contaminated with S*almonella* spp. The major prevalent serotypes in the duck slaughterhouses were *S.* Typhimurium, and 4 different serotypes, *S.* London, *S.* Hadar, *S.* Hogton and *S.* Ohio, were consistently found in the chilling waters or carcasses. In the 13 chicken slaughterhouses, *Salmonella* was recovered from 10 slaughterhouses. Three and 2 slaughterhouses had *Salmonella* contamination in only the first chilling water and carcasses, respectively, and 5 slaughterhouses were *Salmonella* positive in all of the process steps from chilling water to carcasses. The major *Salmonella* serotypes in the chicken slaughterhouses were *S.* Enteritidis and *S.* Montevideo isolated from 7 and 4 slaughterhouses, respectively. Two minor different serotypes, *S.* London and *S.* Newport, were found in 2 slaughterhouses.

The characteristics of the *Salmonella* isolates with Am^R are presented in Table 2. A variety of resistance patterns were noted among the Am^R isolates with resistance to non-

Table 1. Distribution and serotypes of *Salmonella* spp. in poultry slaughterhouses

Species	Slaughter house code	Chilling water		Post-chilled carcasses[a)]				
		The 1st	The last	1	2	3	4	5
Duck	A	*S.* Hadar	*S.* Typhimurium	*S.* Typhimurium	*S.* London	*S.* Typhimurium	*S.* Hadar	*S.* Typhimurium
	B	*S.* Typhimurium	*S.* Typhimurium	*S.* Hogton, *S.* Ohio	*S.* Typhimurium	*S.* Typhimurium, *S.* Hadar	*S.* Hadar, *S.* Ohio	*S.* Ohio
Chicken	C	-	-	-	-	-	-	-
	D	*S.* Enteritidis	*S.* Enteritidis	*S.* Enteritidis	*S.* Enteritidis	*S.* Enteritidis	*S.* Enteritidis	*S.* Enteritidis
	E	*S.* Enteritidis	*S.* Enteritidis	*S.* Enteritidis	*S.* Enteritidis	*S.* Enteritidis	*S.* Enteritidis	*S.* Enteritidis
	F	*S.* Montevideo	*S.* Enteritidis	*S.* Montevideo	-	*S.* Montevideo	*S.* Montevideo	*S.* Senftenberg
	G	*S.* Enteritidis	-	-	-	-	-	-
	H	*S.* Enteritidis	-	-	-	-	-	-
	I	*S.* Montevideo	-	-	*S.* Enteritidis	-	-	-
	J	-	-	-	*S.* London	-	*S.* London	-
	K	-	-	*S.* Enteritidis	*S.* Montevideo	*S.* Montevideo	-	*S.* Montevideo
	L	-	-	-	-	-	-	-
	M	-	-	-	-	-	-	-
	N	*S.* Montevideo	-	-	-	-	-	-
	O	*S.* Newport	-	-	-	*S.* Newport	-	-
Total	15	10/15 (66.7)[b)]	5/15 (33.3)			32/75 (42.7)[c)]		

a) Five carcass samples were taken within each slaughterhouse, b) No. of positive/No. of total slaughterhouses sampled (%), c) No. of positive/No. of total carcasses sampled (%).

Table 2. Phenotypes and genotypes in 11 ampicillin resistant *Salmonella* isolates

Serotype	Origin	MICs[a)] (μg/m*l*)			Phenotype of resistance to β-lactams[b)]	Phenotype of resistance to non-β-lactams[b)]	Double Disc synergy test	Genotypes
		Am	Ctx	Caz				
S. Enteritidis	Chilling water	>512	0.25	0.5		NaTeCS	-ve	bla_{TEM}
	Chilling water	>512	0.25	0.5		NaS	-ve	bla_{TEM}, $bla_{CTX\text{-}M\text{-}14}$
	Carcass	>512	0.25	0.5		NaS	-ve	bla_{TEM}
	Carcass	>512	0.25	0.5		NaS	-ve	bla_{TEM}
	Carcass	>512	0.25	0.5	Cf	NaS	-ve	bla_{TEM}
	Carcass	>512	0.25	0.5	Cf	NaS	-ve	bla_{TEM}
	Carcass	>512	0.25	0.5		NaS	-ve	bla_{TEM}
	Carcass	>512	>512	128	CzCfFepCtxCaz	GmKNaTeS	+ve	$bla_{CTX\text{-}M\text{-}3}$
S. Hadar	Chilling water	>512	0.25	0.5	CzCf	KTeS	-ve	bla_{TEM}
	Carcass	>512	0.5	1	CzCf	TeS	-ve	bla_{TEM}
S. London	Carcass	>512	0.125	0.5	Cf	TeS	-ve	bla_{TEM}, $bla_{CTX\text{-}M\text{-}14}$

a) MIC (μg/m*l*) determined via the agar dilution method in accordance with CLSI standards.
b) Am, ampicillin; Ctx, cefotaxime; Caz, ceftazidime; Cf, cephalothin; Cz, cefazolin; C, chloramphenicol; Gm, gentamicin; K, kanamycin; Na, nalidixic acid; Te, tetracycline; S, streptomycin.

β-lactams, Na, S, Te, C, K or Gm. In cross-resistance to other β-lactams, 3 isolates were resistant to Cf, two to Cz and Cf and one to Cz, Cf, Fep, Ctx and Caz. Regarding the presence of β-lactamase gene families, bla_{TEM}, bla_{SHV} and bla_{CTX} genes were screened and sequenced. Eight of the 11 Am^R isolates carried bla_{TEM} only, two carried bla_{TEM} and $bla_{CTX\text{-}M\text{-}14}$ and one carried $bla_{CTX\text{-}M\text{-}3}$. The DDST detected the presence of ESBLs in only one Am^R isolate with the $bla_{CTX\text{-}M\text{-}3}$ β-lactamase gene. This isolate presented a high MIC value for Am (>512 μg/m*l*), Ctx (>512 μg/m*l*) and Caz (128 μg/m*l*) and also showed multi-resistance to Am, Ca, Cf, Fep, Ctx, Caz, Gm, K, Na, Te and S.

The characteristics of the *Salmonella* isolates with Na resistance are presented in Table 3. All 27 *Salmonella* isolates with a high MIC value to Na (≥512 μg/m*l*) had low MIC concentrations (0.25 to 0.5 μg/m*l*) for Cip. To determine if the amino acids were changed in *gyrA* and *parC* associated quinolone resistance, the genes were amplified from the chromosomal DNA of all isolates by PCR and verified by DNA sequencing. A missense mutation in *gyrA* was the only

Table 3. Phenotypes and genotypes in 27 nalidixic acid resistant *Salmonella* isolates

Species	Strain designation	MICs (μg/m*l*)		Amino acid change in[a)]				Phenotypes of resistance to nonquinolones and nonfluoroquinolones[b)]
				*gyr*A mutation		*par*C mutation		
		Nalidixic acid	Ciprofloxacin	S83	D87	S80	D84	
S. Enteritidis	080001	512	0.25	-	G	-	-	AmTeCS
	080005	512	0.5	-	G	-	-	AmS
	080009	512	0.25	-	G	-	-	AmS
	080014	512	0.25	-	G	-	-	AmS
	080018	512	0.5	-	G	-	-	AmCfS
	080022	512	0.5	-	G	-	-	AmCfS
	080025	512	0.5	-	G	-	-	AmS
	080075	>512	0.5	-	N	-	-	-
	080079	>512	0.5	-	Y	-	-	-
	080083	>512	0.5	-	Y	-	-	-
	080088	>512	0.5	-	Y	-	-	-
	080091	>512	0.5	-	Y	-	-	-
	080095	>512	0.5	-	Y	-	-	-
	080100	>512	0.5	-	Y	-	-	-
	080122	512	0.25	-	G	-	-	TeS
	080126	>512	0.5	-	Y	-	-	-
	080135	>512	0.5	-	Y	-	-	-
	080147	>512	0.5	-	N	-	-	GmKAmCzCfFepCtxTeSCaz
S. Montevideo	080103	>512	0.25	-	G	-	-	-
	080108	512	0.25	-	G	-	-	-
	080112	>512	0.25	-	G	-	-	-
	080114	512	0.25	-	G	-	-	-
	080130	512	0.5	-	G	-	-	-
S. Senftenberg	080107	512	0.5	-	N	-	-	-
	080118	512	0.25	-	G	-	-	-
S. Newport	080162	512	0.5	-	Y	-	-	-
	080167	>512	0.5	-	Y	-	-	-

a) S, serine; D, aspartic acid; V, valine; H, histidine; G, glycine; N, asparagine; Y, tyrosine.
b) Am, ampicillin; Te, tetracycline; C, chloramphenicol; S, streptomycin; Cf, cephalothin; Gm, gentamicin; K, kanamycin; Cz, cefazolin; Fep, cefepime; Ctx, cefotaxime; Caz, ceftazidime.

Table 4. Distribution of PFGE profiles and phage types of *S.*Enteritidis and *S.*Typhimurium

Species	PFGE type		No. of isolates	Phage types (No. of isolates included)	Antibiotic resistance pattern[a)] (No. of isolates included)
	*Xba*I	*Bln*I			
S. Enteritidis	XEI	BEI	8	PT32a (5)	Na (5)
				PT35 (2)	Na (2)
				RDNC[b)](1)	Na (1)
	XEII	BEII	4	PT1 (2)	AmNaS (1), AmCfNaS (1)
				PTIc (2)	AmNaS (1), AmCfNaS (1)
	XEII	BEIII	3	PT1c (3)	AmNaS (1), AmCfNaS (1), AmNaTeCS (1)
	XEIII	BEI	1	PT3 (1)	NaTeS (1)
	XEIV	BEI	1	PT1c (1)	Na (1)
	XEV	BEIV	1	PT1c (1)	GmKAmCzCfFepCtxNaTeSCaz (1)
S. Typhimurium	XTI	BTI	7	RNDC (7)[b)]	- (6), TeS (1)
	XTII	BTII	1	RNDC (1)	- (1)

a) Na, nalidixic acid; Te, tetracycline; Cf, cephalothin; Am, ampicillin; K, kanamycin; Gm, gentamicin; C, chloramphenicol; Cz, cefazolin; Ctx, cefotaxime; Fep, cefepime; S, streptomycin; Caz, ceftazidime.
b) RDNC, Reaction dose not conform to any recognized phage types.

mutation found in amino acid codon Asp87 with substitutions of Gly (n=14), Tyr (n=10) and Asn (n=3). No mutations in the QRDR of *parC* were detected.

All phenotypic and genotypic properties of the major

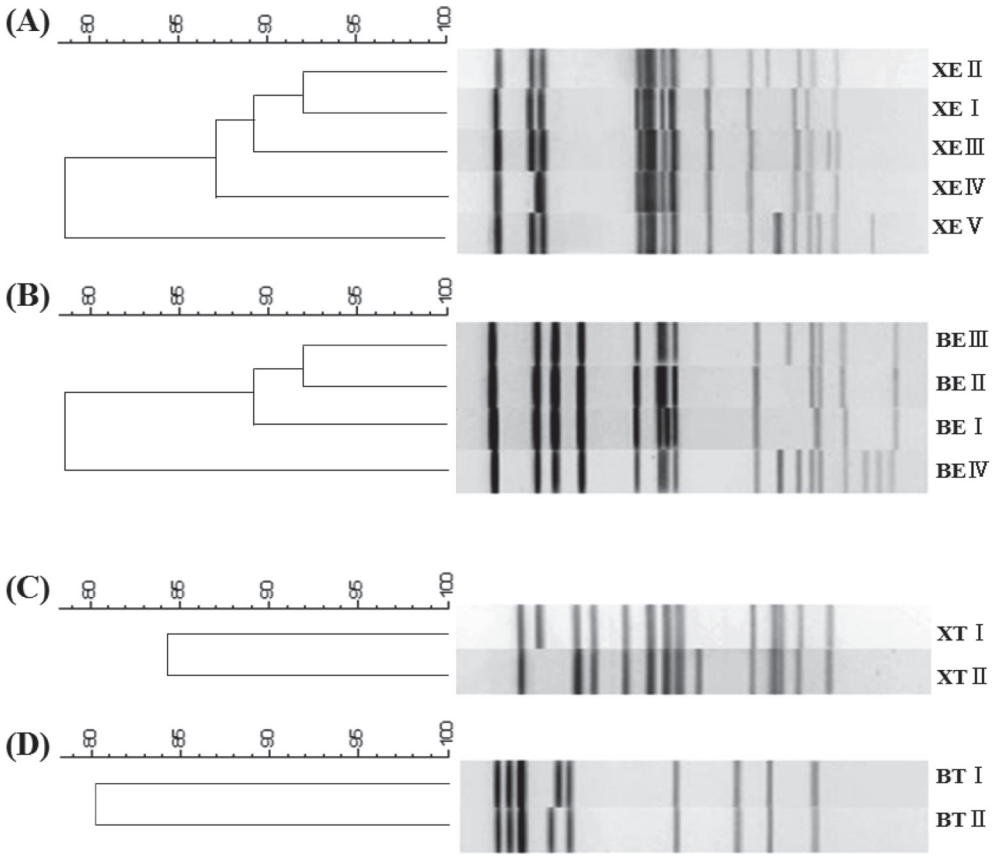

Fig. 1. PFGE patterns of *Salmonella* spp. (A)*S.* Enteritidis digested with the restriction enzyme *Xba*I. (B)*S.* Enteritidis digested with *Bln*I. (C)*S.* Typhimurium digested with *Xba*I. (D)*S.* Typhimurium digested with *Bln*I.

serogroups, 18 *S.* Enteritidis and 8 *S.* Typhimurium, are presented in Table 4. Nine pattern types for *S.* Enteritidis and two for *S.* Typhimurium were identified based on all the properties, PFGE (Fig. 1), phage types and antibiotic resistance pattern. The predominant patterns of *S.* Enteritidis and *S.* Typhimurium were XEI/BEI-PT32a-Na^R (n=5) and XTI/BTI-RNDC-no resistant antibiotics (n=6), respectively.

DISCUSSION

In many countries all over the world including Korea, Japan, U.S.A. and Europe, a wide range of different *Salmonella* serotypes have been found to contaminate the broiler houses, flocks and carcasses of the poultry industry [43]. In this study, 42.7% of the carcasses sampled from 15 poultry slaughterhouses were contaminated with *Salmonella* and showed a higher prevalence than poultry carcasses originating from other countries like Spain (17.9%), Canada (21.2%) and Ireland (26.4%) [1, 7, 14]. Although different sampling procedures, sample sizes and bacterial isolation and identification methods could affect the prevalences of *Salmonella* spp., this elevated level of contamination indicates a potential breakdown of hygiene at various stages at poultry farms and processing plants [22]. Additionally, *S.* Enteritidis and *S.* Typhimurium, which are responsible for most *Salmonella* infections in humans, were the major serotypes in this study, and this result supports that contaminated carcasses are the major source of infection in human Salmonellosis [9].

The level of carcass contamination was less than that of the first chilling water, but more than that of the last chilling water. In adequately controlled chilling systems, microbial contamination of the carcasses is reduced due to the washing effect and hyper-chlorination of the chilling water [4]. Our findings suggest the possibility that pathogenic bacteria within the feather follicles of the carcasses are not easily destroyed by disinfection. However, Buhr *et al*. [6] and Cason *et al*. [8] reported that feather follicles do not harbor bacteria or make only a minor contribution to carcass bacteria populations.

Increased multiple antibiotic resistance has been reported for *Salmonella* spp. isolated from many countries including Korea [9, 16]. Even though ESBLs are less prevalent in *Salmonella*, resistance to third generation cephalosporins in *Salmonella* is of concern, since ESBL-producing *Salmonella* from humans and animals has been isolated in many parts of the world [39, 42]. β-lactamases are capable of conferring bacterial resistance to penicillins, to first-, second- and third-generation cephalosporins and to Atm by hydrolysis of these

antibiotics, which are inhibited by β-lactamase inhibitors, such as clavulanic acid [33].

One previous study [32] reported that the major ESBL was TEM, but SHV was not detected in Am^R *Salmonella* isolates derived from poultry meats and poultry byproducts. The present study also agrees with a previous report [32]; however, the CTX-M-3 and CTX-M-14 types were also detected. Eight of the 11 Am^R isolates carried bla_{TEM} only, two carried bla_{TEM} and $bla_{CTX\text{-}M\text{-}14}$ and one carried $bla_{CTX\text{-}M3}$. These findings show that the bla_{TEM} genes, which have been reported to be the most widely distributed β-lactamase in Korea [37], are the leading types in producing β-lactam resistance in the poultry isolates of *Salmonella* and that CTX-M type β-lactamase is an increasing trend in many countries around the world [31, 45] and play an increasing role in ESBLs in Korea.

DDST detected the presence of ESBLs in one Am^R isolate with the $bla_{CTX\text{-}M\text{-}3}$ β-lactamase gene, and the rate was about 2.0% with a lower proportion than that of the 5.8% attributed to the isolates from poultry and chicken meat in Korea [45] and 2.5% from chicken feces in Spain [39] and 2.5% from chicken meat in Japan [44]. However, our prevalence was higher than the prevalence of approximately 0.8% from poultry flocks in Belgium from 1999 to 2003 [2]. The present study did not investigate the relationship between human isolates and *Salmonella* isolates originating from chickens; however, a previous study in Belgium and France [2] has reported the transmission of *Salmonella* isolates producing the CTX-M-type ESBL in humans through the food chain. Therefore, the appropriate use of antibiotics in both humans and food animals and surveillance programs to monitor antibiotic resistance patterns are essential to control multidrug-resistance in this zoonotic pathogen.

From recent reports, the emergence of quinolone-resistant isolates of *Salmonella* pathogens in humans and veterinary medicine is on the increase [18, 34], and quinolone resistance in *Salmonellae* is mainly associated with mutations in the QRDR of the *gyrA* and *parC* genes [19]. In this study, 27 *Salmonella* isolates carried Asp87 to Gly, Tyr or Asn substitution in *gyrA*. This mutation has been described in *Salmonella* isolates of human and animal origins [18], has been shown to be resistant to Na and has been known to reduce the susceptibility to fluoroquinolones. Although all the isolates in this study showed no mutations in Ser83 of the *gyrA* genes, Griggs *et al*. [18] and Liebana *et al*. [30] obtained high percentages of mutations in *Salmonella* isolates from different animals. This study also confirmed that *parC* mutations are not necessary to obtain a high level of resistance to Na which is in agreement with previous reports [38, 41].

Phage typing is an appropriate tool for epidemiological surveillance of *Salmonella enterica* serovar Enteritidis and Typhimurium in various countries [20]. In this study, PTIc and PT32a were the common phage types of *S.* Enteritidis. This result suggests that the major phage types of *S.* Enteritidis from chickens in Korea have changed since 2002. Woo [46] reported that the major phage type of *S*. Enteritidis isolated from domestic poultry and humans in 2002 was PT4. However, PT4 has not appeared in the literature since that time [11, 28, 29]. Recently, Kang *et al*. [24] reported PT1 and PT21 accounted for 27.2% and 20.8%, respectively; however, PT4 has been found in only 8.7%. This shows that PT4 is not considered the main domestic phage type in Korea.

Some previous studies [24, 27] showed that the PFGE patterns of *S.* Enteritidis in South Korea are similar even when the isolates have different phage types. The present investigation also confirmed that 18 *S.* Enteritidis isolates with different phage types displayed similar PFGE patterns in agreement with previous reports [24, 27].

This study shows that the domestic serotypes are S. Enteritidis and S. Typhimurium in chicken and duck slaughterhouses in Korea, respectively and provides detailed information about *Salmonella* isolates from poultry in Korea. In addition, the appearance of multiple resistant *Salmonella* isolates from poultry suggests the need for a more prudent use of antibiotics and the importance of controlling this pathogen in poultry products.

REFERENCES

1. Arsenault, J., Letellier, A., Quessy, S. and Boulianne, M. 2007. Prevalence and risk factors for *Salmonella* and *Campylobacter* spp. carcass contamination in broiler chickens slaughtered in Quebec, Canada. *J. Food Prot.* **70**: 1820–1828. [Medline]
2. Bertrand, S., Weill, F. X., Cloeckaert, A., Vrints, M., Mairiaux, E., Praud, K., Dierick, K., Wildemauve, C., Godard, C., Butaye, P., Imberechts, H., Grimont, P. A. and Collard, J. M. 2006. Clonal emergence of extended-spectrum beta-lactamase (CTX-M-2)-producing *Salmonella enterica* serovar Virchow isolates with reduced susceptibilities to ciprofloxacin among poultry and humans in Belgium and France (2000 to 2003). *J. Clin. Microbiol.* **44**: 2897–2903. [Medline] [CrossRef]
3. Biendo, M., Canarelli, B., Thomas, D., Rousseau, F., Hamdad, F., Adjide, C., Laurans, G. and Eb, F. 2008. Successive emergence of extended-spectrum beta-lactamase-producing and carbapenemase-producing *Enterobacter aerogenes* isolates in a university hospital. *J. Clin. Microbiol.* **46**: 1037–1044. [Medline] [CrossRef]
4. Blank, G. and Powell, C. 1995. Microbiological and hydraulic evaluation of immersion chilling for poultry. *J. Food Prot.* **58**: 1386–1388.
5. Brooks, J. T., Matyas, B. T., Fontana, J., DeGroot, M. A., Beuchat, L. R., Hoekstra, M. and Friedman, C. R. 2012. An outbreak of *Salmonella* serotype *Typhimurium* infections with an unusually long incubation period. *Foodborne Pathog. Dis.* **9**: 245–248. [Medline] [CrossRef]
6. Buhr, R. J., Cason, J. A., Dickens, J. A., Hinton, A. Jr. and Ingram, K. D. 2000. Influence of flooring type during transport and holding on bacteria recovery from broiler carcass rinses before and after defeathering. *Poult. Sci.* **79**: 436–441. [Medline]
7. Capita, R., Alonso-Calleja, C. and Prieto, M. 2007. Prevalence of *Salmonella enterica* serovars and genovars from chicken carcasses in slaughterhouses in Spain. *J. Appl. Microbiol.* **103**: 1366–1375. [Medline] [CrossRef]
8. Cason, J. A., Hinton, A. Jr. and Buhr, R. J. 2004. Impact of feathers and feather follicles on broiler carcass bacteria. *Poult. Sci.* **83**: 1452–1455. [Medline]
9. Cheong, H. J., Lee, Y. J., Hwang, I. S., Kee, S. Y., Cheong, H.

W., Song, J. Y., Kim, J. M., Park, Y. H., Jung, J. H. and Kim, W. J. 2007. Characteristics of non-typhoidal *Salmonella* isolates from human and broiler-chickens in southwestern Seoul, Korea. *J. Korean Med. Sci.* **22**: 773–778. [Medline] [CrossRef]
10. Chia, J. H., Chu, C., Su, L. H., Chiu, C. H., Kuo, A. J., Sun, C. F. and Wu, T. L. 2005. Development of a multiplex PCR and SHV melting-curve mutation detection system for detection of some SHV and CTX-M beta-lactamases of *Escherichia coli, Klebsiella pneumoniae*, and *Enterobacter cloacae* in Taiwan. *J. Clin. Microbiol.* **43**: 4486–4491. [Medline] [CrossRef]
11. Choi, S. H., Lee, B. K., Kim, N. J., Jeong, J. Y., Ryu, J. and Kim, Y. S. 2005. Increasing incidence of quinolone resistance in human non-typhoid *Salmonella enterica* isolates in Korea and mechanisms involved in quinolone resistance. *J. Antimicrob. Chemother.* **56**: 1111–1114. [Medline] [CrossRef]
12. Chung, Y. H., Kim, S. Y. and Chang, Y. H. 2003. Prevalence and antibiotic susceptibility of *Salmonella* isolated from foods in Korea from 1993 to 2001. *J. Food Prot.* **66**: 1154–1157. [Medline]
13. Clinical and Laboratory Standards Institute (CLSI). 2007. Performance standards for antimicrobial susceptibility testing; 17th Informational Supplement. CLSI Document M100-S17. Clinical and Laboratory Standards Institute, Wayne.
14. Duffy, G., Cloak, O. M., O'Sullivan, M. G., Guillet, A., Sheridan, J. J., Blair, I. S. and McDowell, D. A. 1999. The incidence and antibiotic resistance profiles of *Salmonella* spp. on Irish retail meat products. *Food Microbiol.* **16**: 623–631. [CrossRef]
15. Eaves, D. J., Liebana, E., Woodward, M. J. and Piddock, L. J. 2002. Detection of *gyrA* mutations in quinolone-resistant *Salmonella enterica* by denaturing high-performance liquid chromatography. *J. Clin. Microbiol.* **40**: 4121–4125. [Medline] [CrossRef]
16. Evans, S. and Davies, R. 1996. Case control study of multiple-resistant *Salmonella typhimurium* DT104 infection of cattle in Great Britain. *Vet. Rec.* **139**: 557–558. [Medline]
17. Gast, R. K. 1994. Understanding *Salmonella enteritidis* in laying chickens: the contributions of experimental infections. *Int. J. Food Microbiol.* **21**: 107–116. [Medline] [CrossRef]
18. Griggs, D. J., Gensberg, K. and Piddock, L. J. 1996. Mutations in *gyrA* gene of quinolone-resistant *Salmonella* serotypes isolated from humans and animals. *Antimicrob. Agents Chemother.* **40**: 1009–1013. [Medline]
19. Hao, H., Yang, B., Shi, J., Xi, M., Wang, X., Cui, Y. and Meng, J. 2011. Drug resistance and related genes of chickenborne *Salmonella* to quinolone and fluoroquinolones. *Wei Sheng Wu Xue Bao.* **51**: 1413–1420. [Medline]
20. Hickman-Brenner, F. W., Stubbs, A. D. and Farmer, J. J. 3rd. 1991. Phage typing of *Salmonella enteritidis* in the United States. *J. Clin. Microbiol.* **29**: 2817–2823. [Medline]
21. Janmohamed, K., Zenner, D., Little, C., Lane, C., Wain, J., Charlett, A., Adak, B. and Morgan, D. 2011. National outbreak of *Salmonella Enteritidis* phage type 14b in England, September to December 2009: case-control study. *Euro Surveill.* **16**. Pii: 19840.
22. Jørgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D. R., Bolton, F. J., Frost, J. A., Ward, L. and Humphrey, T. J. 2002. Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. *Int. J. Food Microbiol.* **76**: 151–164. [Medline] [CrossRef]
23. Jung, S. C., Song, S. W., Kim, S. I., Jung, M. E., Kim, K. H., Lee, J. Y. and Lim, S. K. 2007. Surveillance of antimicrobial susceptibility of bacteria isolated from slaughterhouse in Korea 2. Antimicrobial susceptibility of *Salmonella* spp. isolated from carcasses in slaughterhouse. *Kor. J. Vet. Publ. Hlth.* **31**: 51–56.
24. Kang, Z. W., Jung, J. H., Kim, S. H., Lee, B. K., Lee, D. Y., Kim, Y. J., Lee, J. Y., Won, H. K., Kim, E. H. and Hahn, T. W. 2009. Genotypic and phenotypic diversity of *Salmonella enteritidis* isolated from chickens and humans in Korea. *J. Vet. Med. Sci.* **71**: 1433–1438. [Medline] [CrossRef]
25. Kim, A., Lee, Y. J., Kang, M. S., Kwag, S. I. and Cho, J. K. 2007a. Dissemination and tracking of *Salmonella* spp. in integrated broiler operation. *J. Vet. Sci.* **8**: 155–161. [Medline] [CrossRef]
26. Kim, S., Choi, Y. G., Eom, J. W., Oh, T. J., Lee, K. S., Kim, S. H., Lee, E. T., Park, M. S., Oh, H. B. and Lee, B. K. 2007b. An outbreak of *Salmonella enterica* serovar Othmarschen at a funeral service in Guri-si, South Korea. *Jpn. J. Infect. Dis.* **60**: 412–413. [Medline]
27. Kim, S. H., Kim, S., Chun, S. G., Park, M. S., Park, J. H. and Lee, B. K. 2008. Phage types and pulsed-field gel electrophoresis patterns of *Salmonella enterica* serovar Enteritidis isolated from humans and chickens. *J. Microbiol.* **46**: 209–213. [Medline] [CrossRef]
28. Kwag, S. I., Bae, D. H., Cho, J. K., Lee, H. S., Ku, B. G., Kim, B. H., Cho, G. J. and Lee, Y. J. 2008. Characteristics of persistent *Salmonella Enteritidis* strains in two integrated broiler chicken operations of Korea. *J. Vet. Med. Sci.* **70**: 1031–1035. [Medline] [CrossRef]
29. Lee, Y. J., Kim, H. J., Park, C. K., Kim, K. S., Bae, D. H., Kang, M. S., Cho, J. K., Kim, A. R., Kim, J. W. and Kim, B. H. 2007. Characterization of *Salmonella* spp. isolated from an integrated broiler chicken operation in Korea. *J. Vet. Med. Sci.* **69**: 399–404. [Medline] [CrossRef]
30. Liebana, E., Clouting, C., Cassar, C. A., Randall, L. P., Walker, R. A., Threlfall, E. J., Clifton-Hadley, F. A., Ridley, A. M. and Davies, R. H. 2002. Comparison of *gyrA* mutations, cyclohexane resistance, and the presence of class I integrons in *Salmonella enterica* from farm animals in England and Wales. *J. Clin. Microbiol.* **40**: 1481–1486. [Medline] [CrossRef]
31. Miriagou, V., Tassios, P. T., Legakis, N. J. and Tzouvelekis, L. S. 2004. Expanded-spectrum cephalosporin resistance in non-typhoid Salmonella. *Int. J. Antimicrob. Agents.* **23**: 547–555. [Medline] [CrossRef]
32 Olesen, I., Hasman, H. and Aarestrup, F. M. Prevalence of beta-lactamases among ampicillin-resistant Escherichia coli and Salmonella isolated from food animals in Denmark. 2004. *Microb. Drug Resist.* **10**: 334–340. [Medline] [CrossRef]
33. Philippon, L. N., Naas, T., Bouthors, A. T., Barakett, V. and Nordmann, P. 1997. OXA-18, a class D clavulanic acid-inhibited extended-spectrum beta-lactamase from *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **41**: 2188–2195. [Medline]
34. Piddock, L. J. 2002. Fluoroquinolone resistance in *S*almonella serovars isolated from humans and food animals. *FEMS Microbiol. Rev.* **26**: 3–16. [Medline]
35. Pitout, J. D., Thomson, K. S., Hanson, N. D., Ehrhardt, A. F., Moland, E. S. and Sanders, C. C. 1998. beta-Lactamases responsible for resistance to expanded-spectrum cephalosporins in *Klebsiella pneumoniae, Escherichia coli*, and *Proteus mirabilis* isolates recovered in South Africa. *Antimicrob. Agents Chemother.* **42**: 1350–1354. [Medline]
36. Popoff, M. Y. and Le Minor, L. 1997. Antigenic formulas of the Salmonella serovars, 7th Revision. World Health Organization Collaborating Centre for Reference and Research on Salmonella. Pasteur Institute, Paris.
37. Rayamajhi, N., Kang, S. G., Lee, D. Y., Kang, M. L., Lee, S. I., Park, K. Y., Lee, H. S. and Yoo, H. S. 2008. Characterization of

TEM-, SHV- and AmpC-type beta-lactamases from cephalosporin-resistant Enterobacteriaceae isolated from swine. *Int. J. Food Microbiol.* **124**: 183–187. [Medline] [CrossRef]
38. Reche, M. P., García de los Ríos, J. E., Jiménez, P. A., Rojas, A. M. and Rotger, R. 2002. *gyrA* Mutations associated with nalidixic acid-resistant *salmonellae* from wild birds. *Antimicrob. Agents Chemother*. **46**: 3108–3109. [Medline] [CrossRef]
39. Riaño, I., Moreno, M. A., Teshager, T., Sáenz, Y., Domínguez, L. and Torres, C. 2006. Detection and characterization of extended-spectrum beta-lactamases in *Salmonella enterica* strains of healthy food animals in Spain. *J. Antimicrob. Chemother*. **58**: 844–847. [Medline] [CrossRef]
40. Ribot, E. M., Fair, M. A., Gautom, R., Cameron, D. N., Hunter, S. B., Swaminathan, B. and Barrett, T. J. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella,* and *Shigella* for PulseNet. *Foodborne Pathog. Dis*. **3**: 59–67. [Medline] [CrossRef]
41. Ruiz, J., Castro, D., Goñi, P., Santamaria, J. A., Borrego, J. J. and Vila, J. 1997. Analysis of the mechanism of quinolone resistance in nalidixic acid-resistant clinical isolates of *Salmonella* serotype *Typhimurium*. *J. Med. Microbiol*. **46**: 623–628. [Medline] [CrossRef]
42. Shahada, F., Chuma, T., Dahshan, H., Akiba, M., Sueyoshi, M. and Okamoto, K. 2010. Detection and characterization of extended-spectrum beta-lactamase (TEM-52)-producing *Salmonella* serotype *Infantis* from broilers in Japan. *Foodborne Pathog. Dis*. **7**: 515–521. [Medline] [CrossRef]
43. Song, Y. K., Shin, D. H., Byun, J. W., Jeon, K. Y. and Jung, B. Y. 2009. Seroprevalence of *Salmonella Pullorum* and *Gallinarum* in grand parent poultry stock farms during the 2006–2007. *Kor. J. Vet. Publ. Hlth.* **33**: 131–136.
44. Taguchi, M., Kawahara, R., Seto, K., Harada, T. and Kumeda, Y. 2012. Extended-spectrum β-lactamase- and AmpC β-lactamase-producing Salmonella enterica strains isolated from domestic retail chicken meat from 2006 to 2011. *Jpn. J. Infect. Dis.* **65**: 555–557. [Medline] [CrossRef]
45. Tamang, M. D., Nam, H. M., Kim, T. S., Jang, G. C., Jung, S. C. and Lim, S. K. 2011. Emergence of extended-spectrum beta-lactamase (CTX-M-15 and CTX-M-14)- producing nontyphoid Salmonella with reduced susceptibility to ciprofloxacin among food animals and humans in Korea. *J. Clin. Microbiol.* **49**: 2671–2675. [Medline] [CrossRef]
46. Woo, Y. K. 2005. Finding the sources of Korean *Salmonella enterica* serovar Enteritidis PT 4 isolates by pulsed-field gel electrophoresis. *J. Microbiol.* **43**: 424–429. [Medline]