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Antibiotic Resistance Patterns and Detection of bla_{DHA-1} in *Salmonella* Species Isolates from Chicken Farms in South Korea▽

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Fifteen nonrepetitive ampicillin-resistant *Salmonella* spp. were identified among 91 *Salmonella* sp. isolates during nationwide surveillance of *Salmonella* in waste from 131 chicken farms during 2006 and 2007. Additional phenotyping and genetic characterization of these 15 isolates by using indicator cephalosporins demonstrated that resistance to ampicillin and reduced susceptibility to cefoxitin in three isolates was caused by TEM-1 and DHA-1 β-lactamases. Plasmid profiling and Southern blot analysis of these three DHA-1-positive *Salmonella* serovar Indiana isolates and previously reported unrelated clinical isolates of DHA-1-positive *Salmonella* serovar Montevideo, *Klebsiella pneumoniae*, and *Escherichia coli* from humans and swine indicated the involvement of the large-size plasmid. Restriction enzyme digestion of the plasmids from the transconjugants showed variable restriction patterns except for the two *Salmonella* serovar Indiana isolates identified in this study. To the best of our knowledge, this is the first report of the presence of the DHA-1 gene among *Salmonella* spp. of animal origin.

Nontyphoidal *Salmonella* (NTS) strains are a significant cause of gastrointestinal infections of food origin. These microbes are a heterogeneous group of medically important Gram-negative bacteria and can infect a wide range of animals, including humans (3, 6, 9–11, 25).

Currently, no antimicrobial therapies are recommended for the treatment of NTS infection unless a patient is of extreme age, has an underlying disease, or is infected with an invasive *Salmonella* sp. However, the use of antibiotics in treatment of clinical enteric infection has been heavily compromised by emerging multidrug-resistant microbes (4, 17, 18, 23). In particular, resistance due to extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases is of special concern as these enzymes confer resistance to some of the front-line antibiotics used to treat enteric infection in humans and animals (4, 13, 14, 19).

Four classes of β-lactamases are known to confer resistance to β-lactam antibiotics. Among these, plasmid-mediated class A and class C β-lactamases have been frequently reported, whereas class B and class D β-lactamases are relatively rare (4). TEM and SHV enzymes of class A β-lactamases are generally found in Gram-negative bacteria and are derived by one or more amino acid substitutions around the active site of the enzyme that is responsible for the ESBL phenotype (4). Recently, the CTX-M enzyme of class A β-lactamases has been increasingly reported from enteric microbes, like *Salmonella* and *Escherichia coli* (4, 5, 9, 15). These have greater activity against cefotaxime than do other oxyimino-β-lactam substrates, like ceftazidime, ceftriaxone, or cefepime (4, 5). Plasmid-mediated AmpC β-lactamases, like DHA and CMY, are not inhibited by clavulanic acid and have been isolated from a wide variety of clinical and community-acquired microbes (2, 4, 13, 14, 16). These β-lactamases are native to the chromosomes of many Gram-negative bacilli but are missing in some genera, like *Salmonella* (4). The majority of β-lactamases reported in *Salmonella* to date have been derived from human clinical isolates, and only limited information is available regarding *Salmonella* spp. derived from farm animals, although isolates from both humans and animals are of clinical and epidemiological importance (4, 15, 25).

In light of this knowledge gap, our study focused on assessing the distribution of *Salmonella* serovars in poultry farms in South Korea. Subsequently, isolates were analyzed for resistance to antibiotics commonly used in farms. Phenotypic and genetic characteristics of ampicillin-resistant *Salmonella* isolates were tested to gain insight into what β-lactamases were prevalent among these strains. We also characterized DHA-1-associated plasmids in these *Salmonella* spp. and compared them with clinical isolates of *Salmonella*, *Klebsiella pneumoniae*, and *Escherichia coli* from humans and from swine.

MATERIALS AND METHODS

Bacterial strains. A total of 91 *Salmonella* isolates were included in the present study. The isolates were collected from May 2006 to September 2007 in the course of a nationwide surveillance that included six provinces (Gyeonggi, Chungbuk, Chungnam, Jeonbuk, Jeonnam, and Jeju). The surveillance was carried out in an effort to identify *Salmonella* isolates from chicken farms ($n = 131$) by the National Veterinary Research and Quarantine Service (NVRQS) of South Korea. Isolates were recovered from egg shells, boots, water, and feed samples (5 g) preenriched in 45 ml buffered peptone water (Difco Co., Franklin Lakes, NJ), at 37°C for 18 h. Then, 0.1 ml of the culture was transferred into 10 ml of Rappaport-Vassiliadis broth (RV broth; Merck & Co., Inc., NJ) and was incubated overnight at 42°C. The RV broth samples were streaked onto Ramback

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agar (Merck & Co.) and incubated overnight at 37°C. Suspected colonies were confirmed to be *Salmonella* spp. by using Vitek (Vitek System; bioMérieux, Marcy l'Etoile, France). All *Salmonella* isolates were serotyped by the Kauffman-White scheme using *Salmonella* O and H antisera according to the procedures described by the manufacturers (BD, MD; Denka Seiken, Tokyo, Japan). Once identified, each bacterial culture was maintained in Trypticase soy broth (TSB; Difco) and mixed with glycerin (20%) (Shinyo Pure Chemicals Co. Ltd., Japan) and stored at −80°C for further analysis.

DHA-1-positive *Salmonella enterica* serovar Montevideo clinical isolates kindly provided by K. Y. Lee (9), single samples of *E. coli* and *K. pneumoniae* isolated from swine (20), and two *K. pneumoniae* clinical isolates (10252 and 10255) obtained from the Korean Type Culture Collection (KTCC), all of which contain the DHA-1 gene, were also included in this study.

Antimicrobial susceptibility test. MICs were determined by broth microdilution methods according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (7). The antimicrobial agents used were ampicillin, amoxicillin, apramycin, ceftiofur, cephalothin, chloramphenicol, ciprofloxacin, enrofloxacin, florfenicol, gentamicin, nalidixic acid, neomycin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control experiments. The MIC was defined as the lowest concentration that completely inhibited growth. The MIC_{50} and MIC_{90} were defined as the antimicrobial concentrations that inhibited growth of 50 and 90% of the strains, respectively.

Isolates showing reduced susceptibility to ampicillin and/or ceftiofur were further investigated for ESBL phenotypes by double-disk diffusion tests using three indicator cephalosporins—cefotaxime, ceftazidime, and cefoxitin alone and in combination with amoxicillin-clavulanic acid (8). MICs were determined for the isolates showing complete or decreased inhibition zone diameters for these antimicrobials.

PCR and sequencing of β-lactamase genes. We targeted class A (TEM, SHV, and CTXM) β-lactamases, AmpC β-lactamases as described by Pérez-Pérez and Hanson (19), class D (OXA) β-lactamases, and PSE-1 β-lactamases that have been reported to contribute to cephalosporin resistance in South Korea (9, 17, 21, 24). Oligonucleotide primer sets and PCR conditions used for amplification of all of these genes except CTX-M (5′-CTGGAGAAAAGCAGCGGAG-3′ and 5′-GTAAGCTGACGCAACGTCCTG-3′; GenBank accession no. FJ424733) and OXA-F (5′-ATGAAAAACACAATACATATCAAC-3′ and 5′-TTCCTGT AAGTGCGGACAC-3′; GenBank accession no. J02967) were as described in our previous studies (21, 22). *E. coli* (E/123), *K. pneumoniae* (K/14), *Salmonella enterica* serovar Typhimurium DT104 (ATCC 2501) (21, 22), and *S.* Montevideo (10) were used as the positive control. Amplified PCR products of expected sizes were subjected to direct sequencing by an automatic sequencer and dye termination sequencing system (Macrogen Co., South Korea). A BLAST search for homologous sequences was conducted in the GenBank database at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/BLAST).

Conjugation assays. Mixed broth culture mating was performed as described previously with *E. coli* J53AzR (resistant to azide) as a recipient strain to observe the transferability of cefoxitin resistance (20). Briefly, a single colony of the donor and recipient strains grown in TSB were mixed and incubated at 37°C for 20 h. MacConkey agar supplemented with sodium azide (200 μg/ml) and cefoxitin (2 μg/ml) was used to select for transconjugants.

Plasmid profiling and DNA hybridization. A single colony of DHA-1-producing strains (poultry, humans, and swine) and their transconjugants that were confirmed by PCR was picked from MacConkey agar plates and cultured overnight in TSB. Plasmid DNA was extracted using a plasmid midi kit (Qiagen, Valencia, CA) by following the manufacturer's protocol. DNA transfer and hybridization were performed according to the manufacturer's protocol (Ambion Inc., Austin, TX). Plasmid DNA from isolates was electrophoresed through agarose and transferred to a positively charged nylon membrane (GE Healthcare, Little Chalfont, England). The DHA-1 PCR product was labeled using the BrightStar psoralen-biotin nonisotopic labeling kit (Ambion Inc., Austin, Texas) and used as a probe to detect the DHA-1 sequence in the plasmid DNA of isolates. Detection was performed using the BrightStar BioDetect nonisotopic detection kit (Ambion Inc.). Likewise, plasmids from the transconjugants were digested with EcoRI and HindIII restriction enzymes to observe the restriction patterns.

RESULTS

Distribution of *Salmonella* serotypes. In this study, a wide variety of *Salmonella* serotypes were identified among samples from poultry farms in South Korea. Overall, 91 *Salmonella* isolates, representing 17 different serotypes, were identified from four provinces: Gyeonggi, Chungbuk, Chungnam, and Jeonnam. *S. enterica* serovar Enteritidis was the most frequently identified serotype, accounting for 37.3% of the samples. Other frequently identified serotypes included *Salmonella* serovar Virchow (18.6%), followed by *Salmonella* serovars Indiana (9.8%), Give (5.49%), and Typhimurium and Istanbul (4.3%) (Table 1). Complete identity of two *Salmonella* isolates

TABLE 1. Distribution, sources, and antimicrobial resistance phenotypes of *Salmonella* serotypes isolated from chicken farms in South Korea

Serotype	Source(s)	Resistance type[a]	No. of isolates
S. Agona	Feces	No resistance observed	2
S. Albert	Feces	Su-Tc	1
S. Augustenborg	Feces	No resistance observed	1
S. Bareilly	Feces	No resistance observed	2
S. Dessau	Feces	Na	2
S. Enteritidis	Feces, eggshell, dead egg yolk, cloaca, liver, water, environmental dust	Amx-Su-Am-Cm-Na-Sm-Tc	1
		Amx-Su-Am-Na-Sm-Tc	1
		Amx-Su-Am-Na-Sm-Tc-Eno	1
		Cf-Na-Ap-Sm	1
		Na	3
		Su	13
		Su-Cm-Flo-Na-Tm	3
		Su-Na-Sm-Tc	1
		Su-Na-Tm	8
		No resistance observed	2
S. Ferruch	Feces	Su-Na-Sm-Tc-Cip	1
S. Give	Feces	Su-Cm-Tc-Tm	3
		Su-Sm-Tc	2
S. Haardt	Feces	Amx-Su-Am-Na-Neo-Sm-Tc-Tm	2
S. Heidelberg	Feces	Su-Na-Ap-Sm-Tm	1
S. Indiana	Feces	Amx-Su-Am-Cf-Na-Ap-Neo-Sm-Tc-Tm-Cef	
		Amx-Su-Am-Te	2
		Amx-Am-Su-Tm-Cef	1
		Amx-Te	1
		Amx-Neo-Te	1
		No resistance observed	3
S. Infantis	Feces	Sm	1
		No resistance observed	1
S. Istanbul	Feces	Amx-Cf-Sm-Tc	1
		Amx-Te	1
		Neo-Tc	1
		Tc	1
S. Truro	Egg yolk	Su-Na-Gm	1
S. Typhimurium	Feces	Amx-Su	1
		No resistance observed	3
S. Virchow	Feces, water, environmental dust, boats, feed	Su-Na-Sm-Tm	4
		Su-Na-Sm-Tm	8
		Su-Na-Tm	1
		Su-Sm	4
S. Warragul	Feces	Su-Na-Tm	1
Salmonella spp. (B group; 4,5;1)	Feces	No resistance observed	1
Salmonella spp. (C1 group; 7;z10)	Feces	Amx-Su-Am-Cf-Sm-Tc-Tm	1

[a] Ampicillin, Am; amoxicillin, Amx; apramycin, Ap; ceftiofur, Cef; cephalothin, Cf; chloramphenicol, Cm; ciprofloxacin, Cip; enrofloxacin, Eno; florfenicol, Flo; gentamicin, Gm; nalidixic acid, Na; neomycin, Neo; streptomycin, Sm; sulfamethoxazole, Su; tetracycline, Tc; and trimethoprim, Tm.

TABLE 2. Antimicrobial susceptibility of *Salmonella* sp. isolates (n = 91) from chicken farms in South Korea

Antimicrobial agent	MIC (μg/ml)				Resistance (%)
	Range	MIC_{50}	MIC_{90}	Breakpoint	
Amoxicillin	≤2–>1,024	≤2	1,024	32[a]	18.7
Ampicillin	≤4–>1,024	≤4	512	32[a]	16.5
Apramycin	2–64	4	8	32[c]	3.3
Ceftiofur	≤0.25–>64	0.5	1	8[c]	2.2
Cephalothin	≤1–256	4	8	32[a]	4.4
Chloramphenicol	2–>256	4	8	32[a]	5.5
Ciprofloxacin	≤0.25–32	≤0.25	≤0.25	4[a]	1.1
Enrofloxacin	≤0.25–2	≤0.25	1	2[b]	1.1
Florfenicol	2–>128	4	8	32[c]	3.3
Gentamicin	≤0.5–128	≤0.5	1	8[b]	4.4
Nalidixic acid	2–>256	>256	>256	32[a]	63.7
Neomycin	≤1–256	≤1	2	16[c]	5.5
Streptomycin	≤1–>256	8	>256	32[c]	38.5
Sulfamethoxazole	8–>1,024	>1,024	>1,024	512[a]	74.7
Tetracycline	≤1–>256	2	256	16[a]	28.6
Trimethoprim	≤1–256	≤1	≤1	16[a]	4.4

[a] The value was a CLSI (M100-S19; 2009) breakpoint.
[b] The value was a CLSI (M31-A3; 2008) breakpoint.
[c] The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) Report, 2006.

(2.1%) that belonged to serogroups B and C1 could not be determined (Table 1).

Antibiotic resistance phenotypes. Among the drugs tested, resistance was most frequently observed for sulfamethoxazole (74.7%), followed by nalidixic acid (63.7%), streptomycin (38.5%), tetracycline (28.6%), and amoxicillin (18.7%). Infrequent resistance was observed for enrofloxacin and ciprofloxacin (1.1%) (Table 2). The isolates exhibited highest MICs to sulfamethoxazole, with an MIC_{50} and MIC_{90} of >1,024 μg/ml, followed by ampicillin (MIC_{90}, 512 μg/ml) and nalidixic acid and streptomycin (MIC_{90}, >256 μg/ml). Likewise, the lowest MIC_{90} values and resistance rates were exhibited by ciprofloxacin and enrofloxacin followed by ceftiofur (Table 2).

Three out of 15 ampicillin-resistant isolates also exhibited reduced susceptibility to ceftiofur. A disk diffusion test with cefotaxime, ceftazidime, and cefoxitin showed three isolates with a reduced zone diameter for cefoxitin. The zone diameter for the indicator cephalosporins, cefotaxime and ceftazidime, did not change for these isolates in double-disk synergy test performed with amoxicillin-clavulanic acid.

Our study showed that 19.78% of the isolates were resistant to three or more antibiotics, 42.8% were resistant to two antimicrobials tested, and no drug resistance was observed in 14.2% of the *Salmonella* isolates (Table 1).

Analysis of the β-lactamase genes. The bla_{TEM-1} gene was amplified in all ampicillin-resistant isolates (n = 15). The identity of the gene in all these isolates was confirmed by sequencing. In addition to TEM-1, the bla_{DHA-1} gene was amplified in three isolates showing reduced susceptibility to cefoxitin. SHV, CTXM, PSE-1 and OXA β-lactamases were not detected in these ampicillin-resistant *Salmonella* sp. isolates. The phenotypic and genetic characteristics of all the ampicillin-resistant isolates are listed in Table 3.

Transferability of cefoxitin resistance. Conjugation experiments demonstrated the transfer of cefoxitin resistance from six DHA-1-positive isolates (*E. coli* and *K. pneumoniae* isolated from swine [20] and *S.* Montevideo [9], one *K. pneumoniae* [1025; KTCC], and two *S.* Indiana isolates) identified in this study. However, cefoxitin resistance was not transferred by one *K. pneumoniae* isolate (10255; KTCC) and one *S.* Indiana isolate (MIC, 2 μg/ml). The transfer frequency ranged from 1.2×10^{-3} to 4.2×10^{-5}.

Plasmid profiling and DNA hybridization. Plasmid profile analysis showed at least one large-size plasmid common to all of these isolates. Likewise, similar bla_{DHA-1} hybridization patterns were observed among the plasmid DNA extracted from one *E. coli* isolate and one *K. pneumoniae* isolate (swine isolates), one *S.* Montevideo and two *K. pneumoniae* isolates (human isolates), and the three *Salmonella* spp. (poultry isolates) (Fig. 1). Plasmids from the transconjugants treated with EcoRI and HindIII restriction enzymes showed different restriction patterns except for the two *S.* Indiana isolates identified in this study.

DISCUSSION

S. Enteritidis and *S.* Typhimurium are the most frequently reported serovars isolated from humans and animals in South Korea. These two serotypes are also the major causes of *Salmonella*-associated food-borne illnesses in South Korea (6, 9–11). In this study, we found *S.* Enteritidis and *S.* Virchow to be the most frequent serotypes among samples from chicken farms and observed that they were isolated from a wide variety of samples, including egg yolk, egg shell, boots, water, and feed (Table 1). Similarly, *S.* Indiana, *S.* Give, and *S.* Typhimurium were among the 17 different serotypes identified in this study. Our previous study of *Salmonella* showed that *S.* Typhimurium was the most prevalent serotype among swine (22). The prevalence of these serotypes among farm animals could be the reason that *S.* Enteritidis and *S.* Typhimurium are the most common causes of food-borne salmonellosis in South Korea. Similar work on *Salmonella* spp. in the European Union and the United States has described *S.* Typhimurium and *S.* Derby to be the most common *Salmonella* serotypes found on animal farms (1, 3), while in Japan, *S.* Blockley and *S.* Hadar were the most commonly identified serotypes in chicken fecal content

TABLE 3. Antibiogram phenotypes and genotypes of *Salmonella* serovars exhibiting decreased susceptibility to ampicillin and/or indicator cephalosporins

Serovar	MIC (μg/ml)[a]					TEM	Detection of class A, C, and D β-lactamases[b]				
	AMP	CEF	CTX	CAZ	FOX		SHV	CTXM	PSE-1	DHA-1	OXA
S. Enteritidis	1,024	1				TEM-1	−	−	−	−	−
S. Enteritidis	1,024	1				TEM-1	−	−	−	−	−
S. Enteritidis	1,024	0.5				TEM-1	−	−	−	−	−
S. Haardt	1,024	0.5				TEM-1	−	−	−	−	−
S. Haardt	1,024	0.5				TEM-1	−	−	−	−	−
S. Indiana	1,024	0.5				TEM-1	−	−	−	−	−
S. Indiana	1,024	0.5				TEM-1	−	−	−	−	−
S. Indiana	1,024	0.5				TEM-1	−	−	−	−	−
S. Indiana	1,024	0.5				TEM-1	−	−	−	−	−
S. Indiana	1,024	2	0.5	1	2	TEM-1	−	−	−	+	−
S. Indiana	1,024	2	0.5	2	4	TEM-1	−	−	−	+	−
S. Indiana	1,024	64	8	4	4	TEM-1	−	−	−	+	−
S. Istanbul	1,024	0.25				TEM-1	−	−	−	−	−
S. Typhimurium	1,024	0.5				TEM-1	−	−	−	−	−
Salmonella spp. (C1 group; 7; z10)	1,024	0.5				TEM-1	−	−	−	−	−

[a] Ampicillin, AMP; ceftiofur, CEF; cefotaxime, CTX; ceftazidime, CAZ; cefoxitin, FOX.
[b] +, detected; −, not detected.

(1). Adaptation to the local environmental and variation in management practices are possible reasons for the diverse distribution and prevalence of *Salmonella* serovars among countries.

In recent years, emerging multidrug resistance in *Salmonella* has complicated the problem of overcoming salmonellosis in humans as well as in animals. In particular, resistance to cephalosporins and fluoroquinolones in *Salmonella* spp. has limited the available therapeutic options for treatment of clinical salmonellosis (6, 11). This problem has raised great concern regarding the relationship between antimicrobial resistance, antibiotic use in animals, and the transfer of multidrug-resistant *Salmonella* spp. between animals and humans (10, 25).

The increased antimicrobial resistance to sulfamethoxazole (MIC_{50} and MIC_{90}, >1,024 μg/ml; resistance, 74.7%), nalidixic acid (MIC_{50} and MIC_{90}, >256 μg/ml; resistance, 63.7%), and streptomycin (MIC_{50}, 8 μg/ml; MIC_{90}, >256 μg/ml; resistance, 38.5%) in these *Salmonella* isolates reflects the nature of the selective pressures imposed by the use of these antimicrobial compounds in poultry farms (12). Reports show that these antimicrobials have been used extensively for the treatment of infections and as prophylaxis in chicken feed (12). In particular, indiscriminate use of enrofloxacin in treatment of poultry diseases is likely the cause for the high nalidixic acid resistance seen in *Salmonella* isolates of poultry origin (8, 12). Most of these nalidixic acid-resistant *Salmonella* isolates also exhibited MIC_{90} values of ≤0.25 and 1 μg/ml for ciprofloxacin and enrofloxacin, respectively. This drug resistance could be a major public health concern, as fluoroquinolones are important antimicrobial compounds in the treatment of clinical salmonellosis in humans (8).

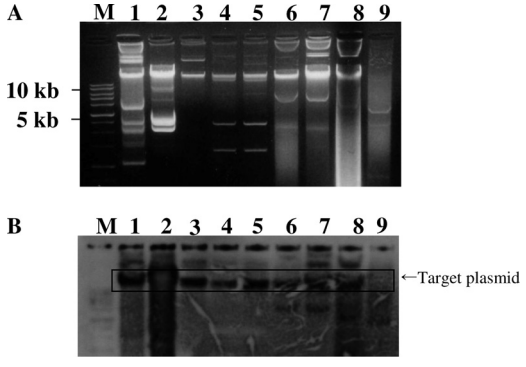

FIG. 1. Plasmid profiling (A) and Southern blot hybridization (B) of DHA-1-positive enteric microbes of human and farm origin. Lane M, 1-kb marker; lane 1, *E. coli* (swine isolate); lane 2, *S.* Montevideo (human isolate); lane 3, *K. pneumoniae* (swine isolate); lanes 4 and 5, *K. pneumoniae* (human isolates); lanes 6, 7, and 8, *S.* Indiana (poultry isolates); lane 9, *S.* Agona (poultry isolates, negative control).

In this study, nine *Salmonella* isolates exhibited ampicillin resistance. PCR and sequencing results showed that this resistance was related to TEM-1 β-lactamase, which has been reported to be the most widely distributed β-lactamase in South Korea (18, 21). However, the percentage of ampicillin-resistant isolates (16.4%) identified in this study was comparatively less than those previously reported for *Salmonella* sp. isolates from swine (64.7%) and humans (39.1%) in South Korea (6, 22). These variations might be due to the nature of antimicrobial selective pressure imposed by the use of different antimicrobials like broad-spectrum cephalosporin (ceftiofur) in swine and extended-spectrum cephalosporin in humans (12, 17, 25).

Among the class C β-lactamases, DHA-1 from clinical enteric microbes of human and animal origin has been reported (9, 16, 17, 21, 23). It was first identified in *S.* Montevideo in 2003 (10). In subsequent years, DHA-1 was reported to be the dominant AmpC β-lactamase among the *Enterobacteriaceae* isolated from hospitals in South Korea (14, 17, 23). However, few data are available on ESBLs or AmpC producers in *Salmonella* from animal origin. In this study, we identified the bla_{DHA-1} gene in three *S.* Indiana isolates. Two isolates were from the same chicken farm, and one was isolated from the farm of a different province. These three *S.* Indiana isolates exhibited reduced susceptibility to the indicator cephalospo-

rins used in the study (Table 3). To our knowledge, this study reports the first bla_{DHA-1} gene among *Salmonella* spp. of animal origin.

The plasmid analysis and hybridization pattern of a DHA-1-specific probe of one *E. coli* isolate and one *K. pneumoniae* (swine isolate), one *S.* Montevideo, and two *K. pneumoniae* (human isolates), and three *S.* Indiana (poultry isolates) isolates indicated that the bla_{DHA-1} gene might be carried on the large-size plasmids of enteric microbes of human and animal origin included in this study (Fig. 1). These plasmids except for the *K. pneumoniae* (10255; KTCC) isolates and one *S.* Indiana isolate (MIC, 2 μg/ml) carrying the bla_{DHA-1} gene were transferable by broth conjugation experiment (10, 21). Restriction enzyme digestion profile of the plasmid from transconjugants of the unrelated clinical isolates from human and farm origin showed different restriction patterns except for the two *S*. Indiana isolates that shared a common restriction pattern for both EcoRI and HindIII. Interestingly, these *S*. Indiana isolates were from the poultry farms located in different provinces. Thus, from these observations, it could be inferred that promiscuous plasmids of variable and closely related types, carrying the bla_{DHA-1} gene, could be in circulation among different clones and serotypes of *Enterobacteriaceae* in South Korea. Our finding is in line with other studies that have reported the involvement of the closely related plasmids carrying ESBL and AmpC genes in different species of *Enterobacteriaceae* (2, 21, 24, 25). However, since the conjugation experiment carried out in this and other previous experiments failed to demonstrate any transfer of plasmid carrying DHA-1 in some of enteric microbes (17, 21), clonal spread and horizontal transfer could be the other possible mechanisms for the spread of DHA-1 β-lactamases among enteric microbes of farm origin.

In summary, our study identified different *Salmonella* spp. among chicken farms in South Korea. This information might be helpful for tracking the sources of food-borne infections and designing preventive measures against nontyphoidal *Salmonella* infection, especially that caused by *S.* Enteritidis, which is an egg-transmitted pathogen of poultry and also has human health implications through consumption of contaminated poultry products. In addition, coexpression of TEM-1 and DHA-1 β-lactamases in *Salmonella* spp. of farm origin may complicate its detection and facilitate the further spread of DHA-1 β-lactamases among zoonotic enteric microbes of farm origin (2, 13, 17, 24).

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