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

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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 (AmR) isolates carried blatEM only, two carried blatEM and blactx-m-14 and one carried blactx-m-1 and only one AmR isolate with the blactx-m-1 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-Na6 (n=5) and XTI/BTI-RNDC-no resistant antibiotics (n=6), respectively.

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


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 ml). 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 ml 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 ml of rinse water was transferred into a
sterile specimen cup.

**Bacterial isolation:** Approximately 25 ml of chilling water or carcass rinse fluid was added to 225 ml BPW and incubated at 35 ± 2°C for 20–24 hr. After pre-enrichment, 0.1 ml of the broth was transferred into a 10 ml 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 antisera (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 (Am, 30 µg), gentamicin (Gm, 10 µg), kanamycin (K, 30 µg), ampicillin (Am, 10 µg), cefazolin (Cz, 30 µg), cephalexin (Ctx, 30 µg), cefotaxime (CtX, 30 µg), cefotaxime (CtX, 30 µg), ceftazidime (Caz, 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 for the following antibiotics: Am, Ctx, Fep, Caz and amoxicillin/clavulanic acid (Amc, 20 /10 µg) as previously described [3].

**PCR detection of the β-lactamase genes:** The presence of genes encoding TEM (forward, 5’-TTCTTGAAGAC-GAAAGGC-3'; reverse, 5'-ACGCTCAGTGGAAC-GAAAC-3'), SHV (forward, 5'-CACTCAAGGATGTATTT-GT-3'; reverse, 5'-TTAGCGTGCCAGTGCTCG-3'), CTX-M-3 group (forward, 5'-AATCAGTGCC-CAGTTCAGCT-3'; reverse, 5'-GAAGCTTCTT-GCTCCAGGCT-3') and CTX-M-14 group (forward, 5'-TACCAGGATAAATACGAGGTG-3'; reverse, 5'-CAGCAGTGTTGTCAGTGCATCC-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’-TGCCTGAGATGGCCCTTGAGGC-3'; reverse, 5'-TACGCTTGATTTATCCACG-3') and parC (forward, 5’-CTATGCGATCTGCGTGG-3'; reverse, 5'-TACACGAGTCGGCGATT-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-typoidal Salmonella by PFGE” [40]. Chromosomal DNA was digested with 50 U of XbaI (Promega, Madison, WI, U.S.A.) or BlnI (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 ml 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 Salmonella 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 found. 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 AmR are presented in Table 2. A variety of resistance patterns were noted among the AmR isolates with resistance to non-
Table 1. Distribution and serotypes of *Salmonella* spp. in poultry slaughterhouses

<table>
<thead>
<tr>
<th>Species</th>
<th>Slaughter house code</th>
<th>Chilling water</th>
<th>Post-chilled carcasses(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>The 1st</td>
<td>The last</td>
</tr>
<tr>
<td>Duck A</td>
<td>S. Hadar</td>
<td>S. Typhimurium</td>
<td>S. Typhimurium</td>
</tr>
<tr>
<td>Duck B</td>
<td>S. Typhimurium</td>
<td>S. Typhimurium</td>
<td>S. Typhimurium</td>
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<td></td>
<td></td>
<td>S. Typhimurium</td>
<td>S. Typhimurium</td>
</tr>
<tr>
<td>Chicken</td>
<td>C</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td></td>
<td>D</td>
<td>S. Enteritidis</td>
<td>S. Enteritidis</td>
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<td></td>
<td></td>
<td>S. Enteritidis</td>
<td>S. Enteritidis</td>
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<td>E</td>
<td>S. Enteritidis</td>
<td>S. Enteritidis</td>
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<tr>
<td></td>
<td></td>
<td>S. Enteritidis</td>
<td>S. Montevideo</td>
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<td>F</td>
<td>S. Montevideo</td>
<td>S. Enteritidis</td>
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<tr>
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<td></td>
<td>S. Montevideo</td>
<td>S. Enteritidis</td>
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<td></td>
<td>G</td>
<td>S. Enteritidis</td>
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<td>-</td>
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<tr>
<td></td>
<td>H</td>
<td>S. Enteritidis</td>
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<td></td>
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<td>-</td>
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<tr>
<td></td>
<td>I</td>
<td>S. Montevideo</td>
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<td>N</td>
<td>S. Montevideo</td>
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<td>O</td>
<td>S. Newport</td>
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<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>15</td>
<td>10/15 (66.7)(^b)</td>
</tr>
</tbody>
</table>

\(a\) 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 (%).

β-lactams, Na, S, Te, C, K or Gm. In cross-resistance to other β-lactams, 3 isolates were resistant to Cz, two to Cz and Cz and one to Cz, Cz, 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*\(^b\) isolates carried *bla*\(_{TEM}\) only, two carried *bla*\(_{TEM}\) and *bla*\(_{CTX, M-14}\) and one carried *bla*\(_{CTX, M-3}\). The DDST detected the presence of ESBLs in only one *Am*\(^b\) isolate with the *bla*\(_{CTX, M-3}\) β-lactamase gene. This isolate presented a high MIC value for Am (>512 µg/ml), Ctx (>512 µg/ml) and Caz (128 µg/ml) and also showed multi-resistance to Am, Ca, Cz, 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/ml) had low MIC concentrations (0.25 to 0.5 µg/ml) 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 2. Phenotypes and genotypes in 11 ampicillin resistant *Salmonella* isolates

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Origin</th>
<th>MICs(^a) (µg/ml)</th>
<th>Phenotype of resistance to β-lactams(^b)</th>
<th>Phenotype of resistance to non-β-lactams(^b)</th>
<th>Double Disc synergy test</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Enteritidis</td>
<td>Chilling water</td>
<td>&gt;512 0.25 0.5</td>
<td>NaTeCS</td>
<td>-</td>
<td><em>bla</em>(_{TEM})</td>
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<tr>
<td></td>
<td>Chilling water</td>
<td>&gt;512 0.25 0.5</td>
<td>NaS</td>
<td>-</td>
<td><em>bla</em>(<em>{TEM}), <em>bla</em>(</em>{CTX, M-14})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carcass</td>
<td>&gt;512 0.25 0.5</td>
<td>NaS</td>
<td>-</td>
<td><em>bla</em>(_{TEM})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carcass</td>
<td>&gt;512 0.25 0.5</td>
<td>NaS</td>
<td>-</td>
<td><em>bla</em>(_{TEM})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carcass</td>
<td>&gt;512 0.25 0.5</td>
<td>Cz</td>
<td>-</td>
<td><em>bla</em>(<em>{TEM}), <em>bla</em>(</em>{CTX, M-3})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carcass</td>
<td>&gt;512 &gt;512 &gt;128</td>
<td>CzCfFepCfxCaz</td>
<td>+ve</td>
<td><em>bla</em>(_{CTX, M-3})</td>
<td></td>
</tr>
<tr>
<td>S. Hadar</td>
<td>Chilling water</td>
<td>&gt;512 0.25 0.5</td>
<td>CzCf</td>
<td>KTeS</td>
<td>-ve</td>
<td><em>bla</em>(_{TEM})</td>
</tr>
<tr>
<td></td>
<td>Carcass</td>
<td>&gt;512 0.5</td>
<td>CzCf</td>
<td>TeS</td>
<td>-ve</td>
<td><em>bla</em>(_{TEM})</td>
</tr>
<tr>
<td>S. London</td>
<td>Carcass</td>
<td>&gt;512 0.125 0.5</td>
<td>CzCf</td>
<td>TeS</td>
<td>-ve</td>
<td><em>bla</em>(<em>{TEM}), <em>bla</em>(</em>{CTX, M-14})</td>
</tr>
</tbody>
</table>

\(a\) MIC (µg/ml) determined via the agar dilution method in accordance with CLSI standards. 
\(b\) Am, ampicillin; Ctx, cefotaxime; Caz, cefazidime; Cf, cephalothin; Cz, cefazolin; C, chloramphenicol; Gm, gentamicin; K, kanamycin; Na, nalidixic acid; Te, tetracycline; S, streptomycin.
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 \textit{parC} were detected.

All phenotypic and genotypic properties of the major
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-NaR (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 Amβ 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 Amβ isolates carried blatem only, two carried blatem and blatCTX-M14 and one carried blatCTX-M3. These findings show that the blatem 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 Amβ isolate with the blatCTX-M3 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 pathogen 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, PT1c 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.

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