Molecular characterization of antimicrobial resistant non-typhoidal *Salmonella* from poultry industries in Korea

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**Abstract**

**Background:** Antimicrobial resistant *Salmonella* strains are a direct threat to human health when this resistance interferes with treatment and an indirect threat when resistance can be transferred to other human pathogens. The objective of the present study was to characterize antimicrobial resistant non-typhoidal *Salmonella* (NTS) isolates recovered from poultry industries, including a description of genetic diversity and virulence profiles.

**Results:** In total of 93 *Salmonella* isolates shown antimicrobial resistance to one or more drugs, all isolates exhibited common resistance to streptomycin, nalidixic acid and cephalothin but no ciprofloxacin resistance. Among 26 virulence gene profiling, 12 virulence genes, *invA, orgA, prgH, sopB, tolC, sipB, gatC, msgA, pagC, spiA, silA*, and *sitC* were found in all antimicrobial-resistant NTS isolates. In comparing the data from ERIC-PCR clusters, virulence profiles and resistance profiles, some *Salmonella* isolates grouped into the same cluster were found to exhibit similar virulence and resistance patterns.

**Conclusions:** Virulence profiling combined with ERIC-PCR offered a rapid approach to characterize antimicrobial-resistant NTS.

**Keywords:** Non-typhoidal Salmonella, Molecular characterization, Resistance

**Background**

Non-typhoidal *Salmonella* (NTS) are major zoonotic food-borne pathogens causing gastroenteritis worldwide. The global burden of NTS infection is estimated to be 93.8 million cases of gastroenteritis each year [30]. In Korea, total of 9,472 human cases of food and water-borne salmonellosis were detected between 1998 and 2007 [25], and NTS strains are one of the most common causes of food poisoning in humans [23]. In many countries including Korea, NTS infections are associated with the consumption of contaminated food products, especially poultry meats and eggs [22, 37].

*Salmonella* virulence genes are present on the bacterial chromosome, plasmids, and prophages, and *Salmonella* pathogenicity islands (SPIs) play important roles in adhesion, invasion, intracellular survival, systemic infection, fimbrial expression, antibiotic resistance, toxin production, and Mg$^{2+}$ and iron uptake [8]. For example, genes such as *invA, orgA, prgH, sipB* and *spaV* in SPI-1 encode a type 3 secretion system 1 (T3SS-1) which allows *Salmonella* to invade phagocytic and non-phagocytic cells. Genes such as *spia* in SPI-2 encode a type 3 secretion system 2 (T3SS-2), which allows the survival and replication of *Salmonella* in host cells [27]. Other chromosomal genes such as *lpfA* and *pefA* encode fimbriae-associated proteins that are important for adherence [11]. Moreover, plasmidal genes such as *spvB* contribute to colonization of deeper tissues, among other functions [2]. The virulence potential of *Salmonella* determines the differences in pathogenicity among *Salmonella* serotypes.

The increase in antibiotic-resistant food-borne pathogens is also a major public health problem. A high rate of antimicrobial-resistance in *Salmonella* strains has been reported in Korea [5, 24]. Antimicrobial resistance and virulence of *Salmonella* strains play an important role in systemic infections with these pathogens [14].

Pulsed-field gel electrophoresis (PFGE) is the gold standard subtyping method used to assess relatedness among *Salmonella* strains from different sources [28]; however it is
Table 1 Distribution of 93 antimicrobial resistant Salmonella isolates derived from poultry industries in Korea

| Salmonella serovars | No | Source | Place | Year | Resistance pattern (n) |
|---------------------|----|--------|-------|------|------------------------|
| S. Agona            | 1  | Feces  | L6    | 2014 | AMP-TE (1)             |
|                     | 1  | Egg belts | L2  | 2013 | CF-NA (1)              |
| S. Bareilly         | 1  | Egg belts | L3  | 2013 | CF-NA-S (1)            |
|                     | 1  | Egg shell | L2  | 2013 | CF-NA (1)              |
|                     | 5  | Egg shell | L3  | 2013 | S (5)                  |
|                     | 1  | Feeder   | L2   | 2013 | S (1)                  |
|                     | 1  | Feces    | L2   | 2013 | S (1)                  |
|                     | 1  | Feces    | L3   | 2013 | S (1)                  |
| S. Binza            | 1  | Chilled duck carcasses | D1 | 2011 | AM-CF-S (1)            |
| S. Braenderup       | 1  | Egg shell | R1  | 2013 | AM (1)                 |
|                     | 1  | Egg shell | R2  | 2013 | AM (1)                 |
|                     | 1  | Feces    | L5   | 2013 | AM (1)                 |
|                     | 1  | Egg shell | L4  | 2013 | AM (1)                 |
|                     | 1  | Dust     | L5   | 2013 | AM (1)                 |
| S. Coquihatiaville  | 1  | Chilled chicken carcasses | C15 | 2011 | C-CF-S (1)            |
| S. Enteritidis      | 1  | Chicken chillers | C2  | 2008 | NA (1)                 |
|                     | 1  | Chicken chillers | C3  | 2008 | NA-S-TE (1)           |
|                     | 2  | Chicken chillers | C4  | 2008 | NA (2)                |
|                     | 2  | Chicken chillers | C5  | 2008 | AM-NA-S (1), AM-C-NA-S-TE (1) |
|                     | 1  | Chilled chicken carcasses | C1  | 2008 | NA (1)                |
|                     | 5  | Chilled chicken carcasses | C4  | 2008 | NA (5)                |
|                     | 5  | Chilled chicken carcasses | C5  | 2008 | AM-NA-S (3), AM-CF-NA-S (2) |
|                     | 1  | Chicken chillers | C6  | 2011 | AM-C-CF-NA-S (1)       |
|                     | 1  | Chicken chillers | C7  | 2011 | CF-NA (1)             |
|                     | 1  | Chicken chillers | C8  | 2011 | CF-NA-S (1)           |
|                     | 2  | Duck chillers | D1   | 2010 | TE (1), CF-S-TE (1)   |
|                     | 1  | Duck chillers | D2   | 2011 | AM-C-CF-NA (1)        |
| S. Give             | 1  | Chilled chicken carcasses | C14 | 2011 | AMP-TE (1)            |
| S. Hadar            | 2  | Duck chillers | D3  | 2008 | AM-CF-S-TE (1), AM-CF-KAN-S-TE (1) |
|                     | 1  | Duck chillers | D9  | 2011 | CF-TE (1)             |
|                     | 1  | Chilled duck carcasses | D1  | 2011 | CF-S-TE (1)           |
|                     | 1  | Chilled duck carcasses | D3  | 2008 | KAN-S (1)             |
|                     | 2  | Chilled duck carcasses | D4  | 2008 | S-TE (2)              |
| S. Hogton           | 1  | Chilled duck carcasses | D4  | 2008 | KAN-S-TE (1)         |
| S. Infantis         | 1  | Chilled duck carcasses | C8  | 2011 | CF-NA-S (1)         |
|                     | 1  | Egg shell  | L1   | 2014 | NA (1)                |
| S. Kortrijk         | 1  | Chicken chillers | C15 | 2011 | C (1)                |
| S. Livingstone      | 1  | Dust       | L7   | 2014 | NA (1)                |
| S. London           | 1  | Chilled duck carcasses | D3  | 2008 | AM-CF-S-TE (1)       |
| S. Malmoe           | 3  | Chicken chillers | C9  | 2011 | S (2), AM-C-CAZ-CF-CTX-NA-TE (1) |
| S. Mbandaka         | 2  | Chilled duck carcasses | D5  | 2010 | CF-S (1), S (1)   |
|                     | 1  | Feces      | L3   | 2013 | CF (1)                |
time consuming and labor intensive [16]. DNA-based fingerprinting techniques such as enterobacterial repetitive intergenic consensus (ERIC), repetitive extragenic palindromic (REP), and BOX repeat-based (BOXAIR) PCR methods are relatively easy to perform, rapid, and sensitive in discriminating between closely related strains [12, 16]. In recent years, the ERIC-PCR fingerprinting method has been used to confirm epidemiological relationships between various isolates, and this method has shown high discriminatory power [1, 32]. The objective of the present study was to characterize antimicrobial-resistant NTS isolates recovered from poultry industries, including a description of genetic diversity and virulence profiles.

Methods

Salmonella isolates

Ninty-three Salmonella isolates showing antimicrobial resistance to one or more drugs were tested in this study. All isolates were recovered from chilled chicken carcasses (n = 25) and chillers (n = 23) in chicken slaughter houses; chilled carcasses (n = 15) and chillers (n = 8) in duck slaughter houses; egg belts (n = 2), feeders (n = 1), feces (n = 5), dust (n = 3) and egg shells (n = 8) from layer farms; and raw shell eggs (n = 2) and egg contents (n = 1) from retail markets between 2008 and 2014 (Table 1). Salmonella isolates were serotyped according to the Kauffmann-White scheme following slide agglutination testing with Salmonella-specific O and H antisera (Difco, Detroit, MI).

Antimicrobial susceptibility tests

Antimicrobial susceptibility profiles of the isolates were determined by the disk diffusion method [6]. Twelve antimicrobial agents (Difco, United States) were tested at the following concentrations: gentamicin (GM, 10 μg), kanamycin (K, 30 μg), cephalothin (CF, 30 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), chloramphenicol (C, 30 μg), ciprofloxacin (CIP, 5 μg),

| Table 1 Distribution of 93 antimicrobial resistant Salmonella isolates derived from poultry industries in KoreaThe 17 chicken slaughter houses, 9 duck slaughter houses, 8 commercial layer farms and 3 retail markets were designated C1 ~ C17, D1 ~ D9, L1 ~ L8 and R1 ~ R3, respectively (Continued) |
|-----------------|-----------------|-------------|---------------|
| S. Montevideo   | 1               | Chicken chillers | C1           | 2008          | NA (1)       |
| S. Montevideo   | 1               | Chicken chillers | C9           | 2008          | NA (1)       |
| S. Montevideo   | 1               | Chicken chillers | C11          | 2011          | CF-NA (1)    |
| S. Montevideo   | 1               | Chicken chillers | C12          | 2011          | CF-NA (1)    |
| S. Montevideo   | 3               | Chilled chicken carcasses | C9   | 2008          | NA (3)       |
| S. Montevideo   | 1               | Chilled chicken carcasses | C10  | 2011          | NA (1)       |
| S. Newbrunswick | 1               | Chilled duck carcasses | D1   | 2011          | S-TE (1)     |
| S. Newport      | 2               | Chicken chillers | C10          | 2011          | NA (2)       |
| S. Newport      | 2               | Chilled chicken carcasses | C10  | 2011          | AM-CF-TE (1), CF-NA (1) |
| S. Ohio         | 1               | Chilled duck carcasses | D4   | 2008          | S-TE (1)     |
| S. Orion        | 1               | Chilled duck carcasses | D8   | 2011          | CF-S (1)     |
| S. Senftenberg  | 1               | Chicken chillers | C9           | 2008          | NA (1)       |
| S. Senftenberg  | 1               | Chicken chillers | C17          | 2011          | CF-NA-S (1)  |
| S. Senftenberg  | 1               | Chilled chicken carcasses | C9   | 2008          | NA (1)       |
| S. Senftenberg  | 1               | Chilled chicken carcasses | C13  | 2011          | NA (1)       |
| S. Senftenberg  | 1               | Chilled chicken carcasses | C16  | 2011          | AM (1)       |
| S. Senftenberg  | 1               | Dust                | L8           | 2014          | GEN-NA-S (1) |
| S. Takoradi     | 2               | Chicken chillers | C9           | 2011          | AM-CF-SXT (1) |
| S. Thomson      | 1               | Chilled chicken carcasses | C7   | 2011          | CF (1)       |
| S. Trachau      | 1               | Egg contents        | R3           | 2013          | CF (1)       |
| S. Typhimurium  | 1               | Chilled chicken carcasses | C10  | 2011          | CTX (1)      |
| S. Typhimurium  | 1               | Duck chillers        | D1           | 2010          | CF-TE (1)    |
| S. Typhimurium  | 1               | Duck chillers        | D3           | 2008          | S-TE (1)     |
| S. Wippra       | 1               | Chilled duck carcasses | D6   | 2011          | S-TE (1)     |
| S. Wippra       | 1               | Chilled duck carcasses | D6   | 2011          | CF-S-TE (1)  |
| Primer | Broad function (gene function) | Sequence (5’ to 3’) | Reference |
|--------|--------------------------------|---------------------|-----------|
| **invA** | Host recognition/invasion (type III secretion system appratus) | F-CTGGCCGCGTGGGGTTTTTGTTGTCTCTCATT<br>R-AGTTTCTCTCTCTTGCAAGTTACCC | Skyberg et al., (2006) [34] |
| **orgA** | Host recognition/invasion (type III secretion system appratus) | F-ATTGTCAGGACAGCCTGAGAGTTGAGAA<br>R-GGCACACGCGGGAACAGGTATT | Skyberg et al., (2006) [34] |
| **prgH** | Host recognition/invasion (type III secretion system appratus) | F-GCCCGACAGCCTGAGAGTTGAGAA<br>R-TGAATATACGGCCGCCCTTGAGCCAGTC | Skyberg et al., (2006) [34] |
| **sopB** | Host recognition/invasion (type III secretion system appratus) | F-CGGACCGCGCCACAAACAAACAGGAGGAAG<br>R-TAGTGATGCCCGTTATGAGTTGATT | Skyberg et al., (2006) [34] |
| **tolC** | Host recognition/invasion (outer membrane channel protein) | F-TACCCAGGCACGAAAAGGAAGAGCTAC<br>R-CCGCCGCTCATACGAGGTTGC | Skyberg et al., (2006) [34] |
| **sopE** | Host recognition/invasion (invasion-associated secreted protein) | F-CATTAGGCCCTTTTCTCTCAGG<br>R-RATGCTGCTGCTGTTATGAGTGATT | Suez et al., (2013) [35] |
| **sil** | Host recognition/invasion (type III secretion system effector protein) | F-CGCCCTATCATAGAACCAG<br>R-CTGCTGACCACTACCTCCC | Suez et al., (2013) [35] |
| **ssek3** | Host recognition/invasion (type III secretion system effector protein) | F-TATCGCTATCTCAAATCATGG<br>R-CGCTTGTATATCATACGGTT | Suez et al., (2013) [35] |
| **sspH1** | Host recognition/invasion (type III secretion system effector protein) | F-GGTCACAGGACACGTTCTACG<br>R-GCGCTTCTTCGTAATTTTCC | Suez et al., (2013) [35] |
| **cdtB** | Host recognition/invasion (toxin-like protein) | F-ACAACTGTCGCATCTCGCCCCGTCATT<br>R-CAATTTGCGTGTTCTGTAGGTGCGAGT | Skyberg et al., (2006) [34] |
| **hlyE** | Host recognition/invasion (hemolysis-inducing protein) | F-GGTCACTGAGGACAGCGTTACGG<br>R-RGCTTTCTTCTTTTTTCC | Suez et al., (2013) [35] |
| **lipC** | Host recognition/invasion (fimbrial protein) | F-GCCCCGCCTGAAGCCTGTGAGT<br>R-CGCGGTACACGCTTACCG | Skyberg et al., (2006) [34] |
| **pefA** | Host recognition/invasion (fimbrial protein) | F-TAAGCCACTGCGAAAGATGC<br>R-GCTGTAACCTCAACACCC | Suez et al., (2013) [35] |
| **tcfA** | Host recognition/invasion (fimbrial protein) | F-TCGCTGATGTTGACTGTTG<br>R-RCCGGCACTCACAACACCG | Suez et al., (2013) [35] |
| **span** | Entry into nonphagocytic cells (type III secretion system appratus) | F-AAAAAGCTTGGAACTGGTTAGTGAAG<br>R-CAGGCCGCTGGAGGATTACCC | Skyberg et al., (2006) [34] |
| **sipB** | Entry into nonphagocytic cells (translocation machinery component) | F-GGACGCCGCCCGGGGAAACTCTC<br>R-ACACTCCCGCGCGCCCTCCACAA | Skyberg et al., (2006) [34] |
| **spiA** | Survival within macrophage (outer membrane secretory protein) | F-CCAGGGGTCTCTTCTCTCTCTGATG<br>R-CGCCGGCAACGCAATGCATCC | Skyberg et al., (2006) [34] |
| **msgA** | Survival within macrophage (macrophage survival protein) | F-GCCAGGGGGCCACGCCCAATCC<br>R-GCGACCGAGCGACATACGCCTCAC | Skyberg et al., (2006) [34] |
| **pagC** | Survival within macrophage (virulence membrane protein) | F-GGCCTCTCTGCGGGGTATG<br>R-GAAGCCGTTTATTTTTTGTAGAGGAGATGTT | Skyberg et al., (2006) [34] |
| **sodC** | Survival within macrophage (periplasmic Cu/Zn superoxide dismutase) | F-ATGGCAGCAGCAAGAG<br>R-AGATGAGAAGTGACGCCTGCAC | Suez et al., (2013) [35] |
| **gctC** | Growth within host (PTS galactitol transporter subunit IIC) | F-ATGGTATTGCTGGTTCTG<br>R-ATCCGCCCGCATAGACGAAC | Suez et al., (2013) [35] |
| **spvB** | Growth within host (ADP-ribo-sylyting toxin) | F-CTATCAGCCCCCGACGAGAAGACGTTTTT<br>R-GGAGGAGGCCGCGTTTGCGCATCATA | Skyberg et al., (2006) [34] |
tetracycline (TE, 30 μg), ampicillin (AM, 10 μg), streptomycin (S, 10 μg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg) and nalidixic acid (NA, 30 μg). An isolate was considered as multidrug-resistant (MDR) when exhibiting resistance to antimicrobials of at least three different classes [29]. *Escherichia coli* strain ATCC 25922 was used as a reference strain.

### Analysis of virulence genes

The DNA for all analyses was extracted by the boiling method [7], and 5 μl of DNA template (approximate 60 ng) was used in each PCR reaction. Primers details are presented in Table 2. To establish the reproducibility of the experiments, PCR reactions were performed twice. The amplified PCR products were visualized by electrophoresis on 1.5% agarose gels stained with ethidium bromide (0.5 μg/ml).

### ERIC-PCR fingerprints analysis

Genotyping of isolates was performed by ERIC-PCR using a pair of primers (F: 5'-ATG TAA GCT CCT GGG GAT TCA C-3'; R: 5'-AAG TAA GTG ACT GGG GTG AGC G-3') [36]. The PCR reaction was performed using a lyophilized PCR master mix according to the manufacturer's instructions. (AccuPower PCR PreMix, Bioneer, Korea). A thermocycler (Bio-Rad, Singapore) was programmed as follows: initial denaturation at 95 °C for 7 min, followed by 30 cycles of denaturation at 90 °C for 30 s, annealing at 52 °C for 1 min and extension at 65 °C for 8 min, and a final extension step at 65 °C for 16 min [39]. A negative control consisting of the same reaction mixture without a DNA template was included in each reaction. ERIC-PCR reactions were repeated at least twice for each isolate to determine the reproducibility of banding patterns. Data were analyzed using the software package BioNumerics 7.5 (Applied Maths, Kistraat, Belgium). A similarity dendrogram was constructed by the UPGMA method with a 1% tolerance limit and 1% optimization, using the DICE similarity coefficient. Clusters were identified based on an 80% similarity cut-off [10]. The discrimination index for ERIC-PCR was calculated using Simpon’s diversity index [19].

### Results

The results of ERIC-PCR, virulence gene profiling, and antimicrobial susceptibility test are summarized in Fig. 1. The 93 *Salmonella* isolates showed resistance to S (n = 46), NA (n = 42), CF (n = 34), AM (n = 24), TE (n = 22), C (n = 6), K (n = 3), CTX (n = 2), CAZ (n = 1), GM (n = 1), and SXT (n = 1). All isolates of *Salmonella* were susceptible to CIP. The 27 isolates (29%) showed multidrug resistance to more than three antibiotic classes.

From the virulence gene profiling, 12 virulence genes, *invA*, *orgA*, *pggH*, *sopB*, *tolC*, *sipB*, *gatC*, *msgA*, *pagC*, *spaI*, *sifA*, and *sitC* were found in all isolates and almost all of the isolates were positive for *spaN* (97%) and *iroN* (97%). However, the other 12 virulence genes, *ssel* (83%), *lpfC* (76%), *sopE* (70%), *hlyE* (60%), *pefA* (55%), *sodC* (48%), *tcfa* (48%), *ssek3* (43%), *STM2759* (26%), *cdtB* (26%), *spvB* (20%) and *ssPH1* (3%) were variably present in the isolates. Especially, *ssPH1* gene was found in only 3 isolates, *S. Montevideo*, *S. Livingstone* and *S. Senftenberg*.

The ERIC-PCR analysis showed that 60 *Salmonella* isolates grouped into 18 clusters (E1 to E18) at 80% genetic similarity, whereas the remaining 33 isolates remained unclustered. The discrimination index of ERIC-PCR typing in this study was 0.974. Except for five clusters (E1, E3, E11, E13 and E14), isolates within each cluster belonged to the same serotype. Irrespective of their sources and places of isolation, all *S. Bareilly* isolates grouped into only one cluster (E12), whereas isolates of other serotypes could be found in different clusters, despite isolation from the same source and location. For example, 18 of 23 *S. Enteritidis* isolates grouped into eight clusters (E3, E4, E5, E7, E8, E10, E11 and E13) and the remaining five isolates of this serotype were not grouped. Two of 8 *S. Montevideo* isolates clustered into E17, and the remaining six isolates were not found in any cluster. Some of these *Salmonella* isolates representing the same serotype, sources, and location clustered separately.

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**Table 2** Primers used in PCR for detection of virulence genes in antimicrobial resistant non-typhoidal *Salmonella* (Continued)

| Primers | Function | Sequence | Source |
|---------|----------|----------|--------|
| sitC    | Iron acquisition (permease) | F-CAGTATATGCTCAACCGCATGTTGCTCC | Skyberg et al., (2006) [34] |
|         |          | R-CGGGGCGAAAAATTAAAGGCCTGTAGTGAAC |        |
| iron    | Iron acquisition (sidrophore) | F-ACTGGCACGCGCTGCCTCGCTCTAT | Skyberg et al., (2006) [34] |
|         |          | R-CGCTTTACCGCCTCTGCCACTGC |        |
| sifA    | Filamentous structure formation (secreted effector protein) | F-TTTGCCAAGGGCCGGCCACAGG | Skyberg et al., (2006) [34] |
|         |          | R-GTTGCCTCTTCCGGCTTTCCACCACTCT |        |
| STM 2759 | Putative dipeptid/oligopeptide/nikel | F-ACCATTTCACCTGGGCTCC | Suez et al., (2013) [35] |
| ABC-type transport systems | R-CGTTCAAGTTTTGTGCCGTTG |        |
Fig. 1 The ERIC-PCR analysis of the non-typhoidal Salmonella from Korean poultry industry is displayed using dendrograms generated by Bionumerics software. The vertical line shows the delineation level of 80%. The red color indicates the presence of the gene while the pink color indicates the absence of the gene. The dark blue color indicates resistance to the corresponding antibiotic while the light blue color indicates susceptibility. The 17 chicken slaughter houses, nine duck slaughter houses, eight commercial layer farms and three retail markets were designated C1 ~ C17, D1 ~ D9, L1 ~ L8, R1 ~ R3, respectively. AM ampicillin, C chloramphenicol, CAZ ceftazidime, CIP ciprofloxacin, CF cephalothin, CTX cefotaxime, GM gentamicin, K kanamycin, NA nalidixic acid, S streptomycin, STX trimethoprim/sulfamethoxazole, TE tetracycline.
Discussion

Antimicrobial-resistant *Salmonella* strains are a direct threat to human health when this resistance interferes with treatment and an indirect threat when resistance can be transferred to other human pathogens [13]. In Korea, *S. Enteritidis*, *S. Montevideo*, *S. Typhimurium* is most common serotypes in poultry slaughter houses [4, 38], and the most frequently observed *Salmonella* serovars in the layer farms were *S. Bareilly* [20].

Cepheus, quinolones and aminoglycosides have been widely used in the poultry industries in Korea [18, 24]. Therefore, *Salmonella* isolates tested in study showed consistent resistance to S, NA, and CF. Our finding showed that 29% among 93 antimicrobial resistance isolates were MDR. All MDR isolates recovered from chicken or duck slaughter houses. However, there was no MDR isolates recovered from layer farms or eggs of retail markets. Generally, *Salmonella* isolated from the broilers demonstrated greater MDR compared to those isolated from layer and eggs [21].

The ability of antimicrobial resistant NTS strains to cause invasive disease can be attributed to various virulence genes, and virulotyping rapidly allows the discrimination of isolates with diverse pathogenetic potential [17]. In this study the genes-invaA, orgA, prgH, sopB, tolC, sipB, gatC, msgA, pagC, spa, sipA, and sitC, located in SPI-1, SPI-2, SPI-5, SPI-11, or others, were found in all antimicrobial-resistant NTS isolates. In addition, spaN, located in SPI-1, and iroN, located in an effector were highly conserved in the isolates. Similar findings were reported in a study of samples from both sick and healthy birds [34], clinical samples, and environmental samples from poultry houses [31], and free-living birds [26].

Recently some researchers have reported that typhoid-associated virulence genes (cdtB, tcfA, and hlyE) in NTS serotypes of human and poultry origin are increasing [11, 26, 34]. NTS strains containing cdtB, tcfA, and hlyE genes were found in this study, and, to our knowledge, this is the first report to detect the presence of cdtB in the *S. Hogton*, *S. Give*, *S. Newbrunswick*, *S. Thomson*, *S. Kortrijk*, *S. Coquillhatville*, and *S. Binza* serotypes.

In this study, lpfC, one of three fimbrial genes, was more prevalent than the others, pefA and tcfA, which is consistent with previous studies [8, 15]. However, no *S. Bareilly* isolates tested in this study harbored the lpfC gene; whereas tcfA was presented in all *S. Bareilly* isolates, and pefA was highly conserved. Gong et al. [15] showed that the presence or absence of specific fimbrial genes in certain *Salmonella* serovars might have diagnostic value, as fimbrial genotypes can be used to determine certain *Salmonella* serovars. Our results of fimbrial gene profiles are consistent with this report.

Molecular typing of *Salmonella* is vital to determining potential sources of infection and implementing effective epidemiological surveillance and control [9]. In this study, 60 out of 93 antimicrobial-resistant NTS isolates grouped into 18 ERIC-PCR clusters, and 33 isolates remained unclustered. This variability might be due to a difference in the sources of samples or in serotypes. In this study, the discrimination index of ERIC-PCR was 0.974. Based on a recommendation by Hunter et al. [19], a D value > 0.9 is desirable for good differentiation; our ability to discriminate between isolates was high. Based on these criteria, ERIC typing is useful for *Salmonella* typing, and our report showed that ERIC-PCR differentiated *Salmonella* strains indistinguishable to levels of heterogeneity of various serotypes.

The distribution of profiles among serotypes demonstrated that different serotypes showed similar fingerprinting patterns. These results are consistent with findings of Ranjbar et al. [33] who found that every *Salmonella* isolate had a unique fingerprinting but the serotypes were not grouped together in major branches.

In this study, the correlations among ERIC-PCR clusters, virulence profiles and resistance profiles were analyzed. We found that virulence genes and resistance profiles correlated with ERIC-PCR subtypes. Some isolates showed the same or similar virulotype or resistance pattern, irrespective of serotypes. The simultaneous presence of a resident virulence plasmid and resistance gene in the same bacterial cell has been reported in *Salmonella* [3]. Therefore, assessing the prevalence of virulence genes as well as the antibiotic resistance status in *Salmonella* serotypes would be useful to better understanding *Salmonella* pathogenicity.

Conclusion

In conclusion, this study provides a molecular characterization of antimicrobial-resistant NTS from poultry industries in Korea. Virulence profiling combined with ERIC-PCR may offers a rapid approach to characterize antimicrobial-resistant NTS isolates. Therefore, determination of their definitive correlations will require future studies with isolates from various source of animal.

Abbreviations

AM: Ampicillin; BOXAIR: BOX repeat-based; C: Chloramphenicol; CAZ: Cefazidime; CF: Cefalothin; CIP: Ciprofloxacin; CLS: Clinical and Laboratory Standards Institute; CTX: Cefotaxime; ERIC: Enterobacterial repetitive intergenic consensus; GM: Gentamicin; K: Kanamycin; MDR: Multidrug-resistant; NA: Nalidixic acid; NTS: Non-typhoidal *Salmonella*; PFGE: Pulsed-field gel electrophoresis; REP: Repetitive extragenic palindromic; S: Streptomycin; SPIs: Pathogenicity islands; SXT: Trimethoprim/sulfamethoxazole; T3SS-1: Type 3 secretion system 1; T3SS-2: Type 3 secretion system 2; TE: Tetracycline

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Authors’ contributions
JKE analyzed the samples, performed the statistical analysis and wrote the manuscript. VJL provided the basic format of the study, obtained the funding and acted as study team leader. Both authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
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