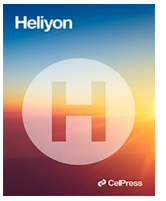
Heliyon 10 (2024) e37124

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

# Nationwide surveillance and characterization of the third-generation cephalosporin-resistant *Salmonella enterica* serovar infantis isolated from chickens in South Korea between 2010 and 2022

Hee-Seung Kang [a], Md Sekendar Ali [a], Seok-Hyeon Na [a], Bo-Youn Moon [a], Ji-In Kim [a], Yu-Jeong Hwang [a], Soon Seek Yoon [a], Seung-Chun Park [b], Suk-Kyung Lim [a,*]

[a] *Bacterial Disease Division, Animal and Plant Quarantine Agency, Gimcheon-si, Republic of Korea*
[b] *Laboratory of Veterinary Pharmacokinetics and Pharmacodynamics, Institute for Veterinary Biomedical Science, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea*

ARTICLE INFO

*Keywords:*
Ceftiofur resistance
$bla_{CTX\text{-}M\text{-}65}$
IncFIB
Pulsotypes
Conjugation

ABSTRACT

The occurrence of extended-spectrum β-lactamase (ESBL)/AmpC β-lactamase-producing *Salmonella* conferring resistance to third-generation cephalosporin has emerged as a global public health concern. In this study, we aimed to investigate the prevalence and molecular characterization of third-generation cephalosporin-resistant *Salmonella enterica* serovar Infantis. In total, 409 *S.* Infatis isolates were collected from the feces and carcasses of healthy and diseased food animals, including chickens (n = 348), pigs (n = 48), cattle (n = 8), and ducks (n = 5) between 2010 and 2022 nationwide in South Korea. Among them, 61.9 % (253/409) of *S.* Infantis strains displayed resistance to ceftiofur, with the most resistant isolates obtained from chickens (98.4 %, 249/253). Moreover, *S.* Infantis isolates showed high resistance (47.7–67.2 %) to streptomycin, ampicillin, nalidixic acid, sulfisoxazole, chloramphenicol, tetracycline, and trimethoprim/sulfamethoxazole. Additionally, the multidrug resistance (MDR) was significantly greater in the ceftiofur-resistant isolates compared to the ceftiofur-susceptible isolates ($p < 0.05$). All the ceftiofur-resistant *S.* Infantis strains produced CTX-M/CMY-2 β-lactamase enzymes, with $bla_{CTX\text{-}M\text{-}65}$ comprising the most (98.4 %, 249/253), followed by $bla_{CTX\text{-}M\text{-}15}$ (1.2 %, 3/253), and $bla_{CMY\text{-}2}$ (0.4 %, 1/253). The ceftiofur-resistant *S.* Infantis belonged to 37 different pulsotypes, with X1A1 (26.1 %, 66/253), X1A2 (20.9 %, 53/253), and X5A3 (9.1 %) being the most prevalent, representing a total of 56.1 % (142/253). Furthermore, the *S.* Infantis sequence type (ST)32 was the most common, accounting for 91.9 % (34/37) of the three distinct STs (ST32, ST16, and ST11) detected across farms located in various provinces nationwide. Most of the $bla_{CMX\text{-}M\text{-}65}$ genes (77.5 %, 193/249), all of the $bla_{CTX\text{-}M\text{-}15}$ genes (100 %, 3/3), and the $bla_{CMY\text{-}2}$ gene (100 %, 1/1) were transferred to the recipient *E. coli* RG488 by conjugation. In addition, the majority of the transconjugants (98.9 %, 191/193) containing $bla_{CTX\text{-}M\text{-}65}$ genes belong to the IncFIB replicon type, playing an important role in the quick and widespread dissemination of *S.* Infantis. Thus,

* Corresponding author. Bacterial Disease Division, Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon-si, 39660, Republic of Korea.
*E-mail address:* imsk0049@korea.kr (S.-K. Lim).

https://doi.org/10.1016/j.heliyon.2024.e37124
Received 2 May 2024; Received in revised form 6 August 2024; Accepted 28 August 2024
Available online 29 August 2024

ceftiofur-resistant *S.* Infantis carrying the β-lactamase genes in chickens has the potential to be transmitted to humans.

## 1. Introduction

The appearance of multidrug-resistant *Salmonella* poses severe human health hazards worldwide [1,2]. Especially third-generation cephalosporin is effectively used for treating invasive salmonellosis in humans and animals [3]. Nevertheless, there has been frequent evidence of cephalosporin-resistant *Salmonella* in both humans and food animals in many countries, including Korea [4], the UK [5], and the USA [6]. The primary cause of resistance to third-generation cephalosporin is the synthesis of extend-spectrum β-lactamase (ESBL) and/or AmpC β-lactamase by bacteria, rendering the cephalosporin ineffective [5]. Hence, ESBL and/or AmpC-carrying *Salmonella* are major concerns for public health worldwide since they limit treatment options in humans [7–9].

*Salmonella enterica* serovar Infantis (*S.* Infantis) is a prevalent serotype of *Salmonella*, often detected in food animals [10]. Among the other food animals, poultry is the primary reservoir for *S.* Infantis [11]. Moreover, *S.* Infantis has been repeatedly identified in humans globally [10]. Of the many other species, *S.* Infantis in poultry recurrently exhibits resistance to non-beta lactam antibiotics, including streptomycin, nalidixic acid, tetracycline, and trimethoprim/sulfamethoxazole [12–14]. In addition, the third-generation cephalosporin-resistant *S.* Infantis has emerged in humans and chickens in many countries [1,15,16]. Moreover, ESBL-harboring *S.* Infantis revealed a link to the pESI (plasmid for emerging *S.* Infantis) plasmid, conferring resistance to third-generation cephalosporin antimicrobial and contributing to their rapid global dissemination [9,17]. In Korea, the prevalence of extended-spectrum cephalosporin-resistant *S.* Infantis in food animals, especially chickens, has been described in some previous investigations [18–20].

In our antimicrobial resistance monitoring program, it was found that the occurrence of *S.* Infantis has suddenly increased in chickens and their products in recent times. To assess the potential risk to humans, it is crucial to understand the phenotypic and molecular characteristics of this serotype in the poultry industry. Thus, the objective of this study was to ascertain the prevalence nationwide and molecular characteristics of ESBL-carrying *S.* Infantis recovered from food animals in South Korea between 2010 and 2022.

## 2. Materials and methods

### 2.1. Salmonella isolation

The isolation, identification, and serotyping of *Salmonella* were accomplished following the previously delineated method [21]. *S.* Infantis strains were obtained from 16 laboratories/centers that took part in the Korean Veterinary Antimicrobial Resistance Monitoring System (KVARS) from 2010 to 2022. The isolation of *Salmonella* species was pre-enrichment in buffered peptone water (Becton Dickinson, CA, USA) and modified semisolid Rappaport Vassiliadis medium (MSRV; Becton Dickinson, CA, USA). The diffused areas at MSRV were cultured on CHROMagar (Merck, Darmstadt, Germany). Identification of the colonies was performed using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (Biomerieux, Marcy L'Etoile, France). The *Salmonella* serogroup was examined by PCR, as described by Ranieri et al. [22]. The confirmation of *S.* Infantis was accomplished using species-specific PCR [23] and agglutination using the White-Kaufmann-Le Minor method [24].

### 2.2. Antimicrobial susceptibility

The antimicrobial sensitivity of the isolates towards the antimicrobials was measured by broth microdilution method using the commercially available Sensititer® panel KRVNF (TREK Diagnostic Systems, WS, UK). The *E. coli* ATCC25922 strain was used as a reference standard. The obtained results were deduced according to the guidelines described by the Clinical and Laboratory Standard Institute (CLSI) [25] and the National Antimicrobial Resistance Monitoring System (NARMS) [26]. Multidrug resistance (MDR) was characterized by the ability of the isolate to resist at least one agent in three or more categories of antimicrobials [27].

### 2.3. Detection of β-lactamase (bla)

The double-disc synergy assessment was conducted to identify the ESBL/AmpC producers among the ceftiofur-resistant isolates utilizing cefotaxime-cefotaxime/clavulanic acid and ceftazidime-ceftazidime/clavulanic acid discs, following the CLSI guidelines [25]. The polymerase chain reaction (PCR) was performed to detect the ceftiofur-resistant *S.* Infantis isolates carrying ESBL/AmpC genes ($bla_{\text{CTX-M}}$, $bla_{\text{CMY-2}}$, $bla_{\text{TEM}}$, and $bla_{\text{SHV}}$), following the previously outlined technique [28]. The primer list and PCR conditions are summarized in Supplementary material (Table S1).

### 2.4. Conjugation assay and replicon typing

The conjugation assay was conducted by the filter-mating technique, using rifampin-resistant *E. coli* RG488 as the recipient strain, following the process described by Ali et al. [29]. Briefly, the donor and recipient strains were subjected to mating at a ratio of 1:4, followed by capturing the bacteria on a membrane filter. The retained bacteria on the filters were incubated, resuspended, and placed

**Table 1**
Prevalence of ceftiofur-resistant *Salmonella enterica* serovar Infantis isolated from food-producing animals between 2010 and 2022 in South Korea.

| Year | Prevalence of ceftiofur-resistant *S.* Infantis % (No. of resistant isolates/No. of tested isolates) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cattle (n = 8) | | | | Pigs (n = 48) | | | | Chickens (n = 348) | | | | Ducks (n = 5) | | | | Total |
| | Feces | Carcasses | Diseased cattle | Subtotal | Feces | Carcasses | Diseased pigs | Subtotal | Feces | Carcasses | Diseased chickens | Subtotal | Feces | Carcasses | Diseased ducks | Subtotal | |
| 2010 | 0 (0/1) | – | – | 0 (0/1) | 0 (0/2) | – | – | 0 (0/2) | – | – | – | 0 (0/0) | – | – | – | 0 (0/0) | 0 (0/3) |
| 2011 | – | – | – | 0 (0/0) | – | – | – | 0 (0/0) | 0 (0/7) | 0 (0/2) | – | 0 (0/9) | – | – | – | 0 (0/0) | 0 (0/9) |
| 2012 | – | 0 (0/4) | – | 0 (0/4) | – | – | – | 0 (0/0) | 0 (0/5) | 0 (0/1) | – | 0 (0/6) | – | – | – | 0 (0/0) | 0 (0/10) |
| 2013 | – | – | – | 0 (0/0) | 0 (0/6) | 0 (0/7) | – | 0 (0/13) | 0 (0/1) | 0 (0/1) | – | 0 (0/2) | – | – | – | 0 (0/0) | 0 (0/15) |
| 2014 | – | – | – | 0 (0/0) | – | – | – | 0 (0/0) | – | 33.2 (2/6) | 0 (0/1) | 28.6 (2/7) | – | – | – | 0 (0/0) | 28.6 (2/7) |
| 2015 | – | – | – | 0 (0/0) | 0 (0/2) | 0 (0/2) | – | 0 (0/4) | – | 0 (0/1) | – | 0 (0/1) | – | – | – | 0 (0/0) | 0 (0/5) |
| 2016 | – | – | – | 0 (0/0) | – | 0 (0/3) | – | 0 (0/3) | – | 0 (0/1) | 0 (0/1) | 0 (0/2) | – | – | – | 0 (0/0) | 0 (0/5) |
| 2017 | – | – | – | 0 (0/0) | 0 (0/1) | – | – | 0 (0/1) | 0 (0/2) | 16.7 (1/6) | – | 12.5 (1/8) | – | – | – | 0 (0/0) | 11.1 (1/9) |
| 2018 | – | – | – | 0 (0/0) | 0 (0/1) | – | – | 0 (0/1) | 0 (0/11) | 0 (0/5) | – | 0 (0/16) | – | 0 (0/1) | 0 (0/2) | 0 (0/3) | 0 (0/20) |
| 2019 | – | – | – | 0 (0/0) | 0 (0/2) | – | 0 (0/1) | 0 (0/3) | – | 0 (0/3) | 0 (0/4) | 0 (0/7) | – | – | – | 0 (0/0) | 0 (0/10) |
| 2020 | – | – | – | 0 (0/0) | 0 (0/5) | 0 (0/6) | – | 0 (0/11) | 71.4 (5/7) | 62.5 (5/8) | 0 (0/1) | 62.5 (10/16) | – | – | – | 0 (0/0) | 37.0 (10/27) |
| 2021 | – | – | 100 (2/2) | 100 (2/2) | 0 (0/1) | 0 (0/3) | – | 0 (0/4) | 78.6 (22/28) | 75.6 (34/45) | 50.0 (2/4) | 75.3 (58/77) | – | 100 (1/1) | – | 100 (1/1) | 72.6 (61/84) |
| 2022 | – | 0 (0/1) | – | 0 (0/1) | – | 0 (0/5) | 0 (0/1) | 0 (0/6) | 90.6 (87/96) | 89.3 (67/75) | 92.3 (24/26) | 90.4 (178/197) | 100 (1/1) | – | – | 100 (1/1) | 87.3 (179/205) |
| Total | 0 (0/1) | 0 (0/5) | 100 (2/2) | 25.0 (2/8) | 0 (0/20) | 0 (0/26) | 0 (0/2) | 0 (0/48) | 72.6 (114/157) | 70.8 (109/154) | 70.3 (26/37) | 71.6 (249/348) | 100 (1/1) | 100 (1/2) | 100 (1/2) | 40 (2/5) | 61.9 (253/409) |

**Table 2**
*Salmonella enterica* serovar Infantis obtained from food-producing animals in different slaughterhouses between 2010 and 2022 in South Korea.

| Slaughter-house | Province /City | 2014 | | 2017 | | 2020 | | 2021 | | 2022 | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of farms | No. of isolates | No. of farms | No. of isolates | No. of farms | No. of isolates | No. of farms | No. of isolates | No. of farms | No. of isolates | No. of farms | No. of isolates |
| A | Gyeonggi | 0 | 0 | 0 | 0 | 2 | 2 | 5 | 11 | 3 | 28 | 10 | 41 |
| B | Chungnam | 0 | 0 | 0 | 0 | 4 | 4 | 7 | 9 | 22 | 26 | 30 | 39 |
| C | Incheon | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 28 | 24 | 28 |
| D | Chungbuk | 2 | 2 | 0 | 0 | 2 | 2 | 1 | 1 | 6 | 9 | 10 | 14 |
| E | Daegu | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 14 | 2 | 9 | 3 | 23 |
| F | Gyeonggi | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 2 | 18 | 3 | 22 |
| G | Gyeongbuk | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 7 | 7 | 9 | 10 | 16 |
| H | Gangwon | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 12 | 6 | 12 |
| I | Jeonbuk | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 3 | 6 | 4 | 7 |
| J | Chungnam | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 2 |
| K | Jeonnam | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 5 | 3 | 5 |
| L | Jeonbuk | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 6 | 0 | 0 | 2 | 6 |
| M | Gyeongnam | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 1 | 3 | 3 |
| N | Gyeonggi | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 4 | 4 | 5 | 5 |
| O | Chungbuk | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 2 | 2 |
| P | Jeonnam | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2 |
| Q | Gyeongnam | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 |
| R | Chungbuk | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| S | Gyeonggi | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 |
| T | Jeonbuk | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 |
| Unknown | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 15 | 21 | 16 | 22 |
| Total | | 2 | 2 | 1 | 1 | 10 | 10 | 29 | 61 | 101 | 179 | 137 | 253 |

onto MacConkey agar plates enriched with rifampin (50 μg/mL) and ceftiofur (25 μg/mL). The selected transconjugants were assessed for the presence of β-lactamase genes and antimicrobial susceptibility patterns. The multiplex PCR was carried out using specific primers and optimal PCR conditions (Table S1) to determine the replicon type from the extracted and purified plasmid DNA, according to the previously described method [28].

### 2.5. Pulsed-field gel electrophoresis and multi-locus sequence typing

The genetic diversity of ESBL/AmpC-harboring isolates was assessed using pulsed-filed gel electrophoresis (PFGE) of the genomic DNA digested with primary enzyme *Xba*I and secondary enzyme *Avr*II (TaKaRa Bio, Inc., Shiga, Japan) [30]. The PFGE band profiles were analyzed using Bionumerics software (version 5.1), and the degree of similarity was assessed using the unweighted pair-group technique with an algorithm-based arithmetic average and Dice similarity index. The clonal relationship of *S.* Infantis was determined using multilocus sequence typing (MLST), following the earlier illustrated method [31]. The housekeeping genes *aroC, dnaN, hemD, hisD, purE, sucA,* and *thrA* were amplified and sequenced in this scheme. In addition, the allelic profile and sequence types (STs) for *S.* Infantis were identified using the web-based MLST database available at https://pubmlst.org/database/.

### 2.6. Statistical analysis

Statistical analysis was conducted using Rex Software (version 3.03, RexSoft Inc., Seoul, Korea). The chi-square test was performed to compare the proportion of resistance between ceftiofur-susceptible and ceftiofur-resistant *S.* Infantis. The $p$-values $<0.05$ were taken into consideration as statistically significant.

## 3. Results

### 3.1. Prevalence of ceftiofur-resistant S. Infantis

We have identified 409 *S.* Infantis isolates from feces and carcass samples of food animals between 2010 and 2022 (Table 1). It was observed that different quantities of isolates were obtained each year. In the first ten years (2010–2019), comparatively fewer numbers of isolates (<5 %) per year were obtained, comprising 22.7 % (93/409). However, the prevalence of *S.* Infantis dramatically increased after 2020, reaching one-fifth in 2021, and half of the total number of isolates was collected in 2022. In terms of animal species levels, the majority of the isolates were obtained from chickens (85.1 %, 348/409) and pigs (11.7 %, 48/409), and a few numbers were collected from cattle (1.9 %, 8/409) and ducks (1.2 %, 5/409). Of the 409 *S.* Infantis isolates, 253 (61.9 %) exhibited resistance to ceftiofur, with the majority of the resistance isolates (98.4 %, 249/253) obtained from chickens. Two isolates of each cattle (25 %, 2/8) and duck (40 %, 2/5) demonstrated resistance to ceftiofur. Overall, the ceftifur resistance proportion varied over the years, starting with resistance in 2014 but gradually increasing from 2020, and reaching its maximum in 2022.

**Table 3**
Antimicrobial resistance of *Salmonella enterica* serovar Infantis isolated from food-producing animals between 2010 and 2022 in South Korea.

| Antimicrobial agents | Range tested (μg/mL) | Break-point (μg/mL) | Comparison between resistance rates of susceptible and resistant isolates to ceftiofur | | | | | | *p*-value | Total *S.* Infantis tested (n = 409) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ceftiofur-susceptible *S.* Infantis (n = 156) | | | Ceftiofur-resistant *S.* Infantis (n = 253) | | | | | | |
| | | | $MIC_{50}$ | $MIC_{90}$ | % Resist. (n) | $MIC_{50}$ | $MIC_{90}$ | % Resist. (n) | | $MIC_{50}$ | $MIC_{90}$ | % Resist. (n) |
| Aminoglycosides | | | | | | | | | | | | |
| Gentamicin | 1–64 | ≥16 | ≤1 | ≤1 | 3.2 (5) | 8 | 8 | 7.1 (18) | 0.2121 | 8 | 8 | 5.6 (23) |
| Streptomycin | 16–128 | ≥32 | ≤16 | ≤16 | 8.3 (13) | 32 | 64 | 93.3 (236) | <0.0001 | 32 | 64 | 60.9 (249) |
| Aminopenicillin | | | | | | | | | | | | |
| Ampicillin | 2–64 | ≥32 | ≤2 | >64 | 12.2 (19) | >64 | >64 | 100 (253) | ND | >64 | >64 | 66.5 (272) |
| β-lactam/β-lactamase inhibitor | | | | | | | | | | | | |
| Amoxicillin/ clavulanic acid | 2/1–32/16 | ≥32/16 | ≤2 | 8 | 0 (0) | ≤2 | 4 | 0.4 (1) | ND | ≤2 | 4 | 0.2 (1) |
| Cephamycin | | | | | | | | | | | | |
| Cefoxitin | 1–32 | ≥32 | 4 | 8 | 0.6 (1) | 16 | 16 | 8.3 (21) | 0.0083 | 8 | 16 | 5.4 (22) |
| Cephalosporin III | | | | | | | | | | | | |
| Ceftiofur | 0.5–8 | ≥8 | 1 | 1 | 0 (0) | >8 | >8 | 100 (253) | ND | >8 | >8 | 61.9 (253) |
| Ceftizidime | 1–16 | ≥16 | ≤1 | ≤1 | 0.6 (1) | 2 | 4 | 1.6 (4) | 0.3938 | 2 | 4 | 1.2 (5) |
| Cephalosporin IV | | | | | | | | | | | | |
| Cefepime | 0.25–16 | ≥16 | ≤0.25 | ≤0.25 | 0 (0) | 2 | 4 | 1.6 (4) | ND | 2 | 4 | 1.0 (4) |
| Fluoroquinolone | | | | | | | | | | | | |
| Ciprofloxacin | 0.12–16 | ≥1 | ≤0.12 | 0.25 | 1.9 (3) | 0.25 | 0.5 | 1.2 (3) | 0.6886 | 0.25 | 0.5 | 1.5 (6) |
| Quinolone | | | | | | | | | | | | |
| Nalidixic acid | 2–128 | ≥32 | 4 | >128 | 14.7 (23) | >128 | >128 | 99.6 (252) | <0.0001 | >128 | >128 | 67.2 (275) |
| Polymyxins | | | | | | | | | | | | |
| Colistin | 2–16 | ≥4 | ≤2 | ≤2 | 0.6 (1) | ≤2 | ≤2 | 0 (0) | ND | ≤2 | ≤2 | 0.2 (1) |
| Folate pathway inhibitors | | | | | | | | | | | | |
| Trimethoprim/ sulfamethoxazole | 0.12/ 2.38–4/76 | ≥4/76 | ≤0.12 | 0.5 | 5.1 (8) | >4 | >4 | 73.9 (187) | <0.0001 | 1 | 4 | 47.7 (195) |
| Sulfisoxazole | 16–256 | ≥512 | 32 | 64 | 9 (14) | >512 | >512 | 96.4 (244) | <0.0001 | >512 | >512 | 63.1 (258) |
| Phenicols | | | | | | | | | | | | |
| Chloramphenicol | 2–64 | ≥32 | 8 | >64 | 13.5 (21) | >64 | >64 | 92.1 (233) | <0.0001 | >64 | >64 | 62.1 (254) |
| Tetracyclines | | | | | | | | | | | | |
| Tetracycline | 2–128 | ≥16 | ≤2 | >128 | 14.1 (22) | >128 | >128 | 99.6 (252) | <0.0001 | >128 | >128 | 67.0 (274) |
| Cabapenem | | | | | | | | | | | | |
| Meropenem | 0.25–4 | ≥4 | ≤0.25 | ≤0.25 | 0 (0) | ≤0.25 | ≤0.25 | 0 (0) | ND | ≤0.25 | ≤0.25 | 0 (0) |
| MDR isolates | | | | | 12.8 (20) | | | 100 (253) | <0.0001 | | | 66.7 (273) |

MIC, minimum inhibitory concentration; $MIC_{50}$ and $MIC_{90}$ are the concentrations (μg/mL) at which 50 % and 90 % of the isolates were inhibited, respectively; MDR, multidrug resistance. ND, non-determined.

### 3.2. Distribution of ceftiofur-resistant S. Infantis isolates

The ceftiofur-resistant *S.* Infantis was widely distributed throughout South Korea. These strains were isolated from 137 poultry farms at 20 slaughterhouses located in 10 different provinces nationwide (Table 2). In note, 60.5 % (153/253) of isolates were recovered from five slaughterhouses (A–C, E, and F). Before 2020, ceftiofur-resistant *S.* Infantis was recovered in only two slaughterhouses located in two provinces. However, the number of isolates dramatically increased in 2021 and 2022 in slaughterhouses in all provinces.

### 3.3. Antimicrobial resistance of S. Infantis

The antibiotic resistance proportion of the *S.* Infantis isolates against the tested antimicrobials is displayed in Table 3. The isolates (>60 %) showed a high proportion of resistance to streptomycin, ampicillin, nalidixic acid, sulfisoxazole, chloramphenicol, and tetracycline, while 47.7 % of the isolates demonstrated resistance to trimethoprim/sulfamethoxazole. In contrast, a very low

**Table 4**
Antimicrobial resistance patterns of *Salmonella enterica* serovar Infantis isolated from food-producing animals between 2010 and 2022 in South Korea.

| No. of Antimicrobials | Ceftiofur-susceptible *S.* Infantis | | Ceftiofur-resistant *S.* Infantis | |
|---|---|---|---|---|
| | No. of isolate (% Resistance) | Most common resistance pattern (No. of isolates) | No. of isolate (% Resistance) | Most common resistance pattern (No. of isolates) |
| 0 | 110 (70.5) | – | – | – |
| 1 | 19 (12.2) | NAL (n = 10) | – | – |
| 2 | 5 (3.2) | AMP CHL (n = 2) | – | – |
| 3 | 10 (6.4) | AMP CHL TET (n = 7) | – | – |
| 4 | 2 (1.3) | NAL STR TET FIS (n = 2) | – | – |
| 5 | 3 (1.9) | AMP CHL CIP NAL TET (n = 2) | 5 (2.0) | AMP XNL CHL NAL TET (n = 4) |
| 6 | 4 (2.6) | CHL NAL STR TET SXT FIS (n = 4) | 12 (4.7) | AMP XNL NAL STR TET FIS (n = 10) |
| 7 | 1 (0.6) | AMP CHL GEN STR TET SXT FIS (n = 1) | 48 (19.0) | AMP XNL CHL NAL STR TET FIS (n = 35) |
| 8 | 1 (0.6) | AMP CHL CIP NAL STR TET SXT FIS (n = 1) | 165 (65.2) | AMP XNL CHL NAL STR TET SXT FIS (n = 150) |
| 9 | – | – | 22 (8.7) | AMP XNL CHL GEN NAL STR TET SXT FIS (n = 14) |
| 10 | 1 (0.6) | AMP FOX CHL GEN NAL STR TET SXT CAZ FIS (n = 1) | 1 (0.4) | AMP XNL CHL GEN NAL STR TET SXT FEP FIS (n = 1) |
| Total | 156 | MDR (n = 20, 14.1 %) | 253 | MDR (n = 253, 100 %) |

AMP, ampicillin; CAZ, ceftizidime; CHL, chloramphenicol; CIP, ciprofloxacin; FEP, cefepime; FIS, sulfisoxazole; FOX, cefoxitin; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; XNL, cefoxitin. MDR, multidrug resistance.

proportion (0.2–5.6 %) of the isolates presented resistance to gentamicin, amoxicillin/clavulanic acid, cefepime, and colistin, whereas all isolates were sensitive to meropenem. In note, it was found that a few isolates showed resistance to ciprofloxacin (MIC ≥1 μg/mL), but the majority demonstrated intermediate resistance to this antimicrobial (MIC ≥0.5 μg/mL). A total of 253 isolates (61.9 %) displayed resistance to ceftiofur; most of these resistant isolates were recovered from chickens (249/253, 98.4 %). In addition, we also compared the resistance proportion between the ceftiofur-resistant group (n = 253) and the ceftiofur-susceptible group (n = 156). The proportion of ceftiofur-resistant isolates showed substantially higher resistance to non-beta lactam antibiotics than that of ceftiofur-susceptible isolates. We observed that streptomycin (8.3 % vs. 93.3 %), nalidixic acid (14.7 % vs. 99.6 %), trimethoprim/sulfamethoxazole (5.1 % vs. 73.9 %), sulfisoxazole (9 % vs. 96.4 %), chloramphenicol (13.5 % vs. 92.1 %), and tetracycline (14.1 % vs. 99.6 %) resistance proportions were considerably higher in the ceftiofur-resistant group compared to the ceftiofur-susceptible group ($p < 0.0001$).

### 3.4. Antimicrobial resistance patterns

The resistance pattern showed a vast difference between the ceftiofur-susceptible and resistant groups. The occurrence of MDR phenotypes was significantly greater in the ceftiofur-resistant isolates than in the ceftiofur-susceptible isolates ($p < 0.0001$). Among the 156 ceftiofur-susceptible *S.* infantis isolates, 70.5 % were susceptible to 15 tested antimicrobials, while the ceftiofur-resistant *S.* Infantis isolates showed multiple resistances (Table 4 and Table S2). Interestingly, among the 253 ceftiofur-resistant isolates, more than half (150/253, 59.3 %) exhibited a similar resistance pattern with ampicillin, ceftiofur, chloramphenicol, nalidixic acid, streptomycin, tetracycline, trimethoprim/sulfamethoxazole, and sulfisoxazole.

### 3.5. Detection of bla genes and transfer of resistance

A total of two $bla_{CTX\text{-}M}$ types and one AmpC β-lactamase gene were detected in 253 ceftiofur-resistant *S.* Infantis isolates. All the ceftiofur-resistant strains were found to produce $bla_{CTX\text{-}M\text{-}65}$ β-lactamases, except four isolates. Three and one isolate carried $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CMY\text{-}2}$, respectively. The conjugation experiment showed that most of the $bla_{CMX\text{-}M\text{-}65}$ genes (77.5 %, 193/249), all of the $bla_{CTX\text{-}M\text{-}15}$ genes (100 %, 3/3), and the $bla_{CMY\text{-}2}$ gene (100%, 1/1) were transferred to the recipient *E. coli* RG488 (Table 5). Furthermore, one more non-β-lactam antimicrobial resistance was transferred with third-generation cephalosporins. Especially tetracycline, chloramphenicol, streptomycin, and trimethoprim/sulfamethoxazole resistance were mostly transferred. Transferred plasmids were identified with IncFIB (98.9 %, 191/193), while the replicon type IncP was detected in one transconjugant among the $bla_{CTX\text{-}M\text{-}65}$-carrying plasmids. Moreover, replicon-type IncFIB was detected in the three transconjugants of $bla_{CTX\text{-}M\text{-}15}$-harboring plasmids. The transconjugant of the $bla_{CMY\text{-}2}$-carrying plasmid contains replicon type IncP and IncK in one isolate (Table 5).

### 3.6. PFGE and MLST of ceftiofur-resistant S. Infantis isolates

In total, 37 diverse pulsotypes were identified in 253 ceftiofur-resistant *S.* Infantis isolates using *Xba*I- and *Avr*II-digested PFGE (Table 6 and Fig. S1). Of them, three common PFGE types were comprised of 56.1 % (142/253): X1A1 (26.1 %), X1A2 (20.9 %), and X5A3 (9.1 %). These types were distributed to nine more slaughterhouses. Specific clones were not continuously distributed; instead, various new types emerged. Although three types (X1A2, X1A4, and X1A11) were persistent during 2020–2022, 18 types emerged in 2022. An X7A12 type was only detected in 2014. A total of 8, 9, and 15 types were detected at A, B, and C slaughterhouses,

**Table 5**
Characteristics of $bla_{CTX-M}$/$bla_{CMY-2}$-carrying *Salmonella enterica* serovar Infantis isolated from food-producing animals between 2010 and 2022 in South Korea.

| Resistance Genes (No. of isolates) | No. of isolates | | | Transferability % (No. of isolates) | Transferred resistance | Replicon type |
|---|---|---|---|---|---|---|
| | Cattle (n = 2) | Chickens (n = 249) | Ducks (n = 2) | | | |
| $bla_{CTX-M-65}$ (n = 249) | 2 | 245 | 2 | 77.5 % (193) | CHL (n = 1) | IncFIB (n = 1) |
| | | | | | CHL STR (n = 2) | IncFIB (n = 2) |
| | | | | | TET CHL (n = 5) | IncFIB (n = 5) |
| | | | | | TET STR (n = 10) | IncFIB (n = 9), IncP (n = 1) |
| | | | | | TET CHL STR (n = 26) | IncFIB (n = 26) |
| | | | | | TET CHL SXT (n = 7) | IncFIB (n = 7) |
| | | | | | TET STR SXT (n = 4) | IncFIB (n = 4) |
| | | | | | TET CHL STR GEN (n = 2) | IncFIB (n = 2) |
| | | | | | TET CHL STR SXT (n = 121) | IncFIB (n = 120), ND (n = 1) |
| | | | | | TET CHL STR SXT GEN (n = 15) | IncFIB (n = 15) |
| $bla_{CTX-M-15}$ (n = 3) | – | 3 | – | 100 % (3) | TET GEN (n = 1), TET STR (n = 2) | IncFIB (n = 1), IncFIB (n = 2) |
| $bla_{CMY-2}$ (n = 1) | – | 1 | – | 100 % (1) | – | IncP, IncK (n = 1) |

CHL, chloramphenicol; GEN, gentamicin; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; ND, not determined.

respectively. Overall, various PFGE types were distributed in different slaughterhouses. We selected one isolate of each PFGE type to determine the sequence type through MLST analysis. A total of three different STs (ST32, ST16, and ST11) were identified among the 37 isolates, with ST32 predominantly detected comprising 91.9 % (34/37). The ST32 was found in $bla_{CTX-M-65}$-carrying *S.* Infantis isolates distributed across numerous farms in various provinces. The remaining ST16 (n = 2) and ST11 (n = 1) were detected in $bla_{CTX-M-15}$-producing isolates.

## 4. Discussion

We found that $bla_{CTX-M-65}$-carrying *S.* Infantis dramatically increased in the Korean chicken industry during 2020–2022. In this study, different numbers of *S.* Infantis isolates were detected in food animals, with the maximum obtained from chickens (85.1 %). Moreover, the prevalence of *S.* Infantis also varied in different years, intensely increasing in 2021 and reaching its maximum (51.1 %) in the final year of the study period in 2022. The prevalence of *S.* Infantis in chickens varied in the different geographical locations, with a higher occurrence found in China (53.5 %) [32], Japan (57.6 %) [33], and Turkey (88.7 %) [23]. In contrast, a comparatively lower incidence was detected in Spain (10.5 %) [34] and Egypt (5.5 %) [35]. This inconsistency could be due to disparities in sample collection and preservation, testing procedures, or period of study [36].

Cephalosporins, particularly third- and fourth-generation, are among the critically important antimicrobials for humans and animals used for treating infections caused by *Enterobacteriaceae,* including *Salmonella* and *E. coli*. However, *Salmonella* strains from livestock have often demonstrated resistance to the third-generation cephalosporin [37]. Previous investigations conducted by our research group found different resistance levels to third-generation cephalosporin in *S.* Virchow (63.8 %) [28], *S.* Albany (3.7 %) [30], and *S.* Typhimurium (3.5 %) [29].

In this study, ceftiofur resistance was identified in 61.9 % of the *S.* Infantis isolates, where the majority of the isolates were obtained from chickens (98.4 %). Similarly, high resistance to extended-spectrum cephalosporin in *S.* Infantis isolated from chickens was identified in the USA (86.2 %) [38], Italy (80.5 %) [13], and Chile (63.2 %) [39]. On the other hand, low levels of cephalosporin-resistant *S.* Infantis isolates from chickens were found in Japan (13.3 %) [40] and Brazil (5.9 %) [41]. In Korea, it was reported that 25 % of the *S.* Infantis strains from retail chicken meat were detected to be resistant to third-generation cephalosporin [4]. Recently, Kim et al. reported a very high prevalence (97.5 %) of third-generation cephalosporin-resistant *S.* Infantis in chickens from integrated broiler operations in Korea [42]. Moreover, there has been an upsurge in the prevalence of ceftiofur-resistant *S.* Infantis in humans in many countries [9,43]. Consequently, the emergence of ceftiofur-resistant *S.* Infantis poses a critical public health concern by limiting the availability of antimicrobials for treating severe infections.

The bacteria that can produce β-lactamases exhibit frequent resistance to other multiple antimicrobials. In this study, it was found that streptomycin, nalidixic acid, trimethoprim/sulfamethoxazole, sulfisoxazole, chloramphenicol, and tetracycline resistance were substantially higher in the ceftiofur-resistant group compared to the ceftiofur-susceptible group. A previous investigation demonstrated that β-lactamase-generating *S.* Typhimurium isolates from food animals possess significant resistance to these antimicrobials [29]. Moreover, various studies have shown that *Salmonella* strains isolated from chickens that produce β-lactamase are resistant to streptomycin, nalidixic acid, trimethoprim/sulfamethoxazole, chloramphenicol, and tetracycline [30,44]. In addition, ceftiofur-resistant *S.* Infantis isolates showed MDR (100 %), consistent with a prior study that found all of the ceftiofur-resistant *S.* Typhimurium isolated from food animals demonstrated MDR phenotypes [29]. Given the multidrug resistance profile observed

**Table 6**
Distribution of pulsotype of *Salmonella enterica* serovar Infantis isolated from food-producing animals between 2010 and 2022 in South Korea.

| Pulsotype | Year | Slaughterhouse (No. of isolate) |
|---|---|---|
| X1A1 (n = 66) | 2021 (n = 28) | E (n = 8), B (n = 7), L (n = 5), A (n = 2), F (n = 1), G (n = 1), M (n = 1), Q (n = 1), S (n = 1), T (n = 1) |
| | 2022 (n = 38) | B (n = 5), C (n = 5), H (n = 5), A (n = 4), D (n = 3), E (n = 2), I (n = 2), K (n = 2), P (n = 1), Unknown (n = 9) |
| X1A2 (n = 53) | 2020 (n = 5) | A (n = 2), O (n = 2), D (n = 1) |
| | 2021 (n = 17) | A (n = 6), G (n = 6), F (n = 3), L (n = 1), N (n = 1) |
| | 2022 (n = 31) | C (n = 7), E (n = 7), B (n = 5), A (n = 3), G (n = 3), D (n = 1), F (n = 1), R (n = 1), Unknown (n = 3) |
| X5A3 (n = 23) | 2022 (n = 23) | A (n = 16), B (n = 3), F (n = 1), P (n = 1), Unknown (n = 2) |
| X1A4 (n = 15) | 2020 (n = 4) | B (n = 4) |
| | 2021 (n = 2) | E (n = 2) |
| | 2022 (n = 9) | H (n = 3), F (n = 2), A (n = 1), C (n = 1), |
| X2A4 (n = 13) | 2022 (n = 13) | F (n = 8), C (n = 4), H (n = 1) |
| X1A3 (n = 11) | 2021 (n = 3) | E (n = 3) |
| | 2022 (n = 8) | C (n = 5), B (n = 2), A (n = 1) |
| X2A2 (n = 11) | 2022 (n = 11) | G (n = 4), F (n = 3), B (n = 2), H (n = 1), Unknown (n = 1) |
| X5A7 (n = 7) | 2021 (n = 1) | J (n = 1) |
| | 2022 (n = 6) | C (n = 3), D (n = 1), N (n = 1), Unknown (n = 1) |
| X5A1 (n = 6) | 2021 (n = 1) | 2022 (n = 5) |
| | 2022 (n = 5) | N (n = 2), B (n = 1), D (n = 1), K (n = 1) |
| X1A7 (n = 6) | 2021 (n = 1) | J (n = 1) |
| | 2022 (n = 5) | D (n = 2), A (n = 1), A (n = 1), N (n = 1) |
| X2A10 (n = 5) | 2022 (n = 5) | B (n = 2), I (n = 2), Unknown (n = 1) |
| X1A11 (n = 4) | 2020 (n = 1) | D (n = 1) |
| | 2021 (n = 1) | M (n = 1) |
| | 2022 (n = 2) | B (n = 1), M (n = 1) |
| X2A1 (n = 4) | 2021 (n = 1) | A (n = 1) |
| | 2022 (n = 3) | A (n = 2), Unknown (n = 1) |
| X1A10 (n = 2) | 2021 (n = 2) | A (n = 2) |
| X2A5 (n = 2) | 2022 (n = 2) | F (n = 2) |
| X5A2 (n = 2) | 2022 (n = 2) | B (n = 2) |
| X7A12 (n = 2) | 2014 (n = 2) | D (n = 2) |
| X11A1 (n = 2) | 2022 (n = 2) | K (n = 2) |
| X1A5 (n = 1) | 2022 (n = 1) | D (n = 1) |
| X1A6 (n = 1) | 2021 (n = 1) | B (n = 1) |
| X1A8 (n = 1) | 2022 (n = 1) | B (n = 1) |
| X1A9 (n = 1) | 2022 (n = 1) | G (n = 1) |
| X2A3 (n = 1) | 2022 (n = 1) | H (n = 1) |
| X2A8 (n = 1) | 2022 (n = 1) | C (n = 1) |
| X3A1 (n = 1) | 2022 (n = 1) | I (n = 1) |
| X3A11 (n = 1) | 2022 (n = 1) | C (n = 1) |
| X4A2 (n = 1) | 2022 (n = 1) | Unknown (n = 1) |
| X4A10 (n = 1) | 2022 (n = 1) | G (n = 1) |
| X5A4 (n = 1) | 2022 (n = 1) | F (n = 1) |
| X5A6 (n = 1) | 2021 (n = 1) | B (n = 1) |
| X5A9 (n = 1) | 2022 (n = 1) | B (n = 1) |
| X6A1 (n = 1) | 2022 (n = 1) | I (n = 1) |
| X8A8 (n = 1) | 2021 (n = 1) | D (n = 1) |
| X9A7 (n = 1) | 2022 (n = 1) | H (n = 1) |
| X10A13 (n = 1) | 2021 (n = 1) | Unknown (n = 1) |
| X12A14 (n = 1) | 2022 (n = 1) | C (n = 1) |
| ND (n = 1) | 2017 (n = 1) | I (n = 1) |

*ND, Not determined.

alongside the detection of $bla_{\text{CTX-M-65}}$, it was anticipated that the MDR might be prompted by the presence of pESI and/or pESI-like plasmids previously described in *S.* infantis [45]. Furthermore, the most frequent MDR patterns of ceftiofur-resistant isolates were chloramphenicol, nalidixic acid, streptomycin, tetracycline, trimethoprim/sulfamethoxazole, and sulfisoxazole, which could restrict the treatment of *S.* Infantis infections.

In our investigation, two different types of $bla_{\text{CTX-M}}$ genes belonging to the ESBL class were detected in the ceftiofur-resistant *S.* Infantis isolates. Among them, the majority was $bla_{\text{CTX-M-65}}$, comprising 98.4 % (249/253), while three isolates (1.2 %, 3/253) carried $bla_{\text{CTX-M-15}}$. The high occurrence of $bla_{\text{CTX-M-65}}$-carrying *S.* Infantis in food animals, especially in chickens, has been found globally, including in Korea [19,42], the USA [15], South America [16,46], and Europe [2,47]. Moreover, $bla_{\text{CTX-M-65}}$-producing *Salmonella* has often been detected in human clinical isolates [2,48]. Recently, an increase in the prevalence of $bla_{\text{CTX-M-65}}$-carrying *S.* Infantis isolated from humans found in the UK [45], the USA [49], and China [50], contributing to the resistance of third-generation cephalosporin antimicrobials. Furthermore, the $bla_{\text{CTX-M-65}}$ gene in *S.* Infantis can be acquired by pESI-like plasmids triggers the resistance to extended-spectrum β-lactam antibiotics [45]. In addition, $bla_{\text{CTX-M-15}}$ is one of the most commonly detected ESBL types in *Salmonella* isolates from humans and food animals worldwide [44,51,52]. In this study, AmpC β-lactamase CMY-2 was detected in one ceftiofur-resistant isolate recovered from chickens. $bla_{\text{CMY-2}}$ is one of the widely spread AmpC types that can provide persistent

resistance to various β-lactams, including third-generation cephalosporin [30,53]. *Salmonella* strains producing $bla_{CMY-2}$ were identified in humans and food animals, including chickens, across various geographical locations [28,54,55]. Moreover, it was shown that ESBL/AmpC-harboring *Salmonella* can be transferred from chickens to humans through direct contact or the food chain, making treatment more complicated [56].

In this study, of the 253 *S.* Infantis isolates producing CTX-M and/or CMY-2 β-lactamase, 193 (76.3 %) isolates producing $bla_{CTX-M-65}$, three isolates producing $bla_{CTX-M-15}$, and one isolate producing $bla_{CMY-2}$ transmitted their β-lactamase genes to the recipient *E. coli* RG488 through conjugation. In a previous investigation, it was found that 40 % of the $bla_{CTX-M-65}$-producing *S.* Infantis isolates obtained from chickens were able to be transferred to recipient bacteria by conjugation [16]. Moreover, $bla_{CTX-M-15}$-carrying *S.* Enteritidis [52] and $bla_{CMY-2}$-producing *S.* Virchow [28] isolated from food animals were also proven to be transferred to the recipient by conjugation in our previous investigations. Genes related to disease development can be transmitted with antimicrobial resistance, allowing ESBL/AmpC genes and pathogenic clusters to move into the plasmids of host bacteria, creating more virulent and multidrug-resistant strains, which can result in challenges for treatment in both humans and animals [7].

The majority of transconjugants carrying the $bla_{CTX-M-65}$ genes belonged to IncFIB in our investigation. A previous study found the presence of the IncFIB plasmid type in *S.* Infantis strains isolated from humans and poultry [9]. The IncFIB conjugative plasmids have significant roles in the quick and widespread dissemination of $bla_{CMX-M-65}$-carrying *Salmonella* [15]. Furthermore, most of the conjugative IncFIB plasmid transconjugants showed non-beta lactam antimicrobial resistance. The high prevalence of one resistant pattern in this study might be due to the presence of an IncFIB-type plasmid. In addition, this result suggested that $bla_{CMX-M-65}$ can be acquired by not only the third-generation cephalosporin but also non-beta lactam antibiotics such as tetracycline, phenicols, streptomycin, and trimethoprim/sulfamethoxazole. Moreover, we identified the coexistence of IncP and IncK plasmids in one $bla_{CMY-2}$-carrying transconjugant. The presence of IncP and IncK plasmids is also associated with the transmission of the $bla_{CMY-2}$ gene in *Enterobacteriaceae* obtained from humans and chickens [17,57].

Our findings suggested that resistance to tetracycline, chloramphenicol, streptomycin, and trimethoprim/sulfamethoxazole was most commonly transferred along with the $bla_{CTX-M-65}$ gene, consistent with a previous investigation that demonstrated *S.* Infantis strains transferring this antimicrobial resistance with the β-lactamase genes [15]. Moreover, the transfer of tetracycline, chloramphenicol, and streptomycin resistance together with β-lactamase genes was detected in *S.* Typhimurium in our previous study in Korea [29]. In addition, it was revealed that horizontal transmission of the $bla_{CTX-M-65}$ gene with *tetA* (the gene for tetracycline), *flo*R (the gene for chloramphenicol), and *aac*(3)-IVa (the gene for streptomycin) in *S.* Infantis was associated with increased resistance to these antimicrobials [49]. However, the antimicrobial resistance, including ciprofloxacin and nalidixic acid, did not transfer to the recipient. It was shown in the previous study that quinolone resistance transfer may not occur due to *gyrA* mutation in the multidrug-resistant *S.* Infantis [9]. Thus, the results suggest that the high resistance proportion of these antimicrobials may be predominately linked to $bla_{CTX-M-65}$-harboring plasmids in ceftiofur-resistant *S.* Infantis isolates.

Due to the limited discriminatory power of PFGE for typing *Salmonella,* we digested the DNA using two restriction enzymes to determine the prevalence of specific clones. The PFGE analysis revealed the presence of 37 distinct pulsotypes, including some new types, among the 253 ceftiofur-resistant *S.* Infantis, with the main clusters identified as three pulsotypes with more than 50 %. The pulsotypes were distributed in different slaughterhouses located in various provinces nationwide, potentially as a result of the widespread distribution of these clones, suggesting their dissemination among the poultry, concurring with the previous study [30]. In addition, numerous previous investigations have demonstrated the widespread dissemination of multidrug-resistant *S.* Infantis clones in humans and food animals globally [10]. Moreover, various new clones emerged in our investigation that could potentially be disseminated to humans and other animals.

Based on the MLST analysis, three distinct STs (ST32, ST16, and ST11) were identified in this investigation. Of them, ST32 was the predominantly detected genotype distributed across numerous farms. Previous research demonstrated the occurrence of multidrug-resistant *S.* Infantis ST32 strains in both humans and poultry [58]. In addition, this clone has been proven to be linked with the cases of salmonellosis in humans transmitted from chickens [46]. Moreover, the additional two *S.* Infantis STs (ST16 and ST11) were found in only $bla_{CTX-M-15}$-carrying and AmpC-producing isolates. However, they also exhibit the potential to infect humans, posing a significant health hazard, as previous research has shown that *Salmonella* strains carrying antimicrobial resistance have been identified with similar STs [59,60].

## 5. Conclusion

The prevalence of ceftiofur-resistant *S.* Infantis obtained from food-producing animals, particularly chickens nationwide in South Korea, has been dramatically increasing since 2020, reaching alarmingly high levels. In addition, most of the isolates exhibited multidrug resistance. Although $bla_{CTX-M-65}$-harboring *S.* Infantis showed various pulsotypes, predominant types were observed in poultry farms. Moreover, ceftiofur-resistant *S.* Infantis carried conjugative plasmids, which showed resistance to various antimicrobials. Thus, clonal and horizontal transmission might play a role in the high prevalence of ceftiofur resistance in the poultry industry. Therefore, it is necessary to implement effective measures, such as decreasing and restricting the use of antimicrobials and hygiene management in farms and slaughterhouses in the poultry sector. Furthermore, additional research is still necessary to detect pESI or pESI-like plasmids in *S.* Infanstis and *gyrA* mutation for quinolone resistance to accurately establish their relationship with multidrug resistance.

## Data availability

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethical statement

No ethical approval was deemed necessary for this study as it did not involve direct experimentation on animals.

## Funding statement

This study was supported by the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food, and Rural Affairs, Republic of Korea [Grant number: N-1543081-2017-24-01].

## CRediT authorship contribution statement

**Hee-Seung Kang:** Writing – original draft, Data curation, Conceptualization. **Md Sekendar Ali:** Writing – original draft, Methodology. **Seok-Hyeon Na:** Methodology. **Bo-Youn Moon:** Writing – review & editing, Methodology, Data curation. **Ji-In Kim:** Methodology. **Yu-Jeong Hwang:** Methodology. **Soon Seek Yoon:** Writing – review & editing. **Seung-Chun Park:** Writing – review & editing, Data curation. **Suk-Kyung Lim:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that there are no competing interests.

## Acknowledgments

We are grateful to the staff of the laboratories/centers participating in the Korean Veterinary Antimicrobial Resistance Monitoring System from 2010 to 2020.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37124.

## References

[1] Y.-S. Liao, H.-L. Wei, H.-C. Kuo, B.-H. Chen, Y.-W. Wang, R.-H. Teng, Y.-P. Hong, J.-H. Chang, S.-Y. Liang, C.-S. Tsao, Chromosome-borne CTX-M-65 extended-spectrum β-lactamase–producing *Salmonella enterica* serovar Infantis, Taiwan, Emerg. Infect. Dis. 29 (2023) 1634.
[2] D. Hindermann, G. Gopinath, H. Chase, F. Negrete, D. Althaus, K. Zurfluh, B.D. Tall, R. Stephan, M. Nüesch-Inderbinen, *Salmonella enterica* serovar Infantis from food and human infections, Switzerland, 2010–2015: poultry-related multidrug resistant clones and an emerging ESBL producing clonal lineage, Front. Microbiol. 8 (2017) 1322.
[3] F. Medalla, W. Gu, B.E. Mahon, M. Judd, J. Folster, P.M. Griffin, R.M. Hoekstra, Estimated incidence of antimicrobial drug-resistant nontyphoidal *Salmonella* infections, United States, 2004-2012, Emerg. Infect. Dis. 23 (2016) 29–37.
[4] H.Y. Jeon, Y. Bin Kim, S.-K. Lim, Y.J. Lee, K.W. Seo, Characteristics of cephalosporin-resistant *Salmonella* isolates from poultry in Korea, 2010–2017, Poultry Sci. 98 (2019) 957–965.
[5] L. Burke, K.L. Hopkins, D. Meunier, E. De Pinna, D. Fitzgerald-Hughes, H. Humphreys, N. Woodford, Resistance to third-generation cephalosporins in human non-typhoidal *Salmonella enterica* isolates from England and Wales, 2010–12, J. Antimicrob. Chemother. 69 (2014) 977–981.
[6] M. Iwamoto, J. Reynolds, B.E. Karp, H. Tate, P.J. Fedorka-Cray, J.R. Plumblee, R.M. Hoekstra, J.M. Whichard, B.E. Mahon, Ceftriaxone-resistant nontyphoidal *Salmonella* from humans, retail meats, and food animals in the United States, 1996–2013, Foodb. Pathog. Dis. 14 (2017) 74–83.
[7] J. Qiao, Q. Zhang, W.Q. Alali, J. Wang, L. Meng, Y. Xiao, H. Yang, S. Chen, S. Cui, B. Yang, Characterization of extended-spectrum β-lactamases (ESBLs)-producing *Salmonella* in retail raw chicken carcasses, Int. J. Food Microbiol. 248 (2017) 72–81.
[8] A.R. Zomorodi, M. Rad, G.R. Hashemitabar, H. Salimizand, Molecular typing of cephalosporin resistant serovars of *Salmonella enterica* from poultry and farm animals, Bulg. J. Vet. Med. 23 (2020) 178–186.
[9] H. Tate, J.P. Folster, C.-H. Hsu, J. Chen, M. Hoffmann, C. Li, C. Morales, G.H. Tyson, S. Mukherjee, A.C. Brown, Comparative analysis of extended-spectrum-β-lactamase CTX-M-65-producing *Salmonella enterica* serovar Infantis isolates from humans, food animals, and retail chickens in the United States, Antimicrob, Agents Chemother. 61 (2017).
[10] D.M. Alvarez, R. Barrón-Montenegro, J. Conejeros, D. Rivera, E.A. Undurraga, A.I. Moreno-Switt, A review of the global emergence of multidrug-resistant *Salmonella enterica* subsp. enterica Serovar Infantis, Int. J. Food Microbiol. (2023) 110297.
[11] N. Nógrády, M. Király, R. Davies, B. Nagy, Multidrug resistant clones of *Salmonella* Infantis of broiler origin in Europe, Int. J. Food Microbiol. 157 (2012) 108–112.
[12] M. Bekoz Yarar, M.K. Turkyilmaz, S. Turkyilmaz, Investigation of Integron, Antimicrobial resistance and virulence gene profiles of *Salmonella enterica* subspecies enterica serovar Infantis isolates obtained from broiler chickens, Isr. J. Vet. Med. 78 (2023) 4.
[13] P.C. Proietti, V. Stefanetti, L. Musa, A. Zicavo, A.M. Dionisi, S. Bellucci, A. La Mensa, L. Menchetti, R. Branciari, R. Ortenzi, Genetic profiles and antimicrobial resistance patterns of *Salmonella* Infantis strains isolated in Italy in the food chain of broiler meat production, Antibiotics 9 (2020) 814.
[14] M. Sin, S. Yoon, Y.B. Kim, E.B. Noh, K.W. Seo, Y.J. Lee, Molecular characteristics of antimicrobial resistance determinants and integrons in *Salmonella* isolated from chicken meat in Korea, J. Appl. Poultry Res. 29 (2020) 502–514.
[15] A.C. Brown, J.C. Chen, L.K.F. Watkins, D. Campbell, J.P. Folster, H. Tate, J. Wasilenko, C. Van Tubbergen, C.R. Friedman, CTX-M-65 extended-spectrum β-lactamase–producing *Salmonella enterica* serotype Infantis, United States, Emerg. Infect. Dis. 24 (2018) 2284.
[16] S. Martínez-Puchol, M. Riveros, K. Ruidias, A. Granda, L. Ruiz-Roldán, C. Zapata-Cachay, T.J. Ochoa, M.J. Pons, J. Ruiz, Dissemination of a multidrug resistant CTX-M-65 producer *Salmonella enterica* serovar Infantis clone between marketed chicken meat and children, Int. J. Food Microbiol. 344 (2021) 109109.

[17] A. Franco, P. Leekitcharoenphon, F. Feltrin, P. Alba, G. Cordaro, M. Iurescia, R. Tolli, M. D'Incau, M. Staffolani, E. Di Giannatale, Emergence of a clonal lineage of multidrug-resistant ESBL-producing *Salmonella* Infantis transmitted from broilers and broiler meat to humans in Italy between 2011 and 2014, PLoS One 10 (2015) e0144802.

[18] D. Choi, J.-W. Chon, H.-S. Kim, D.-H. Kim, J.-S. Lim, J.-H. Yim, K.-H. Seo, Incidence, antimicrobial resistance, and molecular characteristics of nontyphoidal *Salmonella* including extended-spectrum β-lactamase producers in retail chicken meat, J. Food Protect. 78 (2015) 1932–1937.

[19] J.Y. Oh, J.H. Jeong, S.M. Kwak, J.-C. Chae, Complete genome of *bla*$_{CTX-M-65}$-carrying *Salmonella* Infantis strain Z01323CSL0015 isolated from broiler chicken, J. Anim. Sci. Technol. (2024).

[20] E.B. Noh, Y. Bin Kim, H.Y. Jeon, K.W. Seo, S.H. Son, Y.J. Lee, Antimicrobial resistance and genetic diversity of *Salmonella* serotypes recovered from edible pork offal from Korea, Microb. Drug Resist. 25 (2019) 1514–1520.

[21] H.-J. Song, S. Ali, B.-Y. Moon, H.Y. Kang, E.J. Noh, T.-S. Kim, S.-J. Kim, J.-I. Kim, Y.J. Lee, S.-S. Yoon, Antimicrobial resistance profiles and molecular characteristics of *Salmonella enterica* serovar Agona isolated from food-producing animals during 2010–2020 in South Korea, Foodb. Pathog. Dis. (2024).

[22] M.L. Ranieri, C. Shi, A.I. Moreno Switt, H.C. Den Bakker, M. Wiedmann, Comparison of typing methods with a new procedure based on sequence characterization for *Salmonella* serovar prediction, J. Clin. Microbiol. 51 (2013) 1786–1797.

[23] Ö.Ş. Yapicier, B. Sareyyüpoğlu, Prevalence and rapid identification of *Salmonella* Infantis in broiler production in Turkey, Ankara Üniversitesi Vet, Fakültesi Derg. 69 (2022) 1–8.

[24] P.A.D. Grimont, F.-X. Weill, Antigenic formulae of the *Salmonella* serovars, WHO Collab. Cent. Ref, Res. Salmonella 9 (2007) 1–166.

[25] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Seventh Informational Supplement, M100- S25, Clinical and laboratory standards institute, Wayne, PA, 2020.

[26] National Antimicrobial resistance monitoring system (NARMS) integrated report, The National Antimicrobial Resistance Monitoring System: Enteric Bacteria, U. S. food and drug administration, Rockville, MD, USA, 2021.

[27] A.-P. Magiorakos, A. Srinivasan, R.B. Carey, Y. Carmeli, M.E. Falagas, C.G. Giske, S. Harbarth, J.F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance, Clin. Microbiol. Infect. 18 (2012) 268–281.

[28] S.H. Na, D.C. Moon, H.Y. Kang, H.-J. Song, S.-J. Kim, J.-H. Choi, J.W. Yoon, S.-S. Yoon, S.-K. Lim, Molecular characteristics of extended-spectrum β-lactamase/AmpC-producing *Salmonella enterica* serovar Virchow isolated from food-producing animals during 2010–2017 in South Korea, Int. J. Food Microbiol. 322 (2020) 108572.

[29] M.S. Ali, S.-H. Na, B.-Y. Moon, H.Y. Kang, H.-S. Kang, S.-J. Kim, T.-S. Kim, Y.-E. Heo, Y.-J. Hwang, S.S. Yoon, Antimicrobial resistance profiles and molecular characteristics of extended-spectrum β-lactamase-producing *Salmonella enterica* serovar Typhimurium isolates from food animals during 2010–2021 in South Korea, Foodb. Pathog. Dis. (2024).

[30] M.S. Ali, H.-J. Song, B.-Y. Moon, S.-J. Kim, H.Y. Kang, D.C. Moon, Y.-H. Lee, D.-H. Kwon, S.-S. Yoon, S.-K. Lim, Antibiotic resistance profiles and molecular characteristics of *bla*$_{CMY-2}$-carrying *Salmonella enterica* serovar Albany isolated from chickens during 2013–2020 in South Korea, Foodb. Pathog. Dis. (2023).

[31] C. Kidgell, U. Reichard, J. Wain, B. Linz, M. Torpdahl, G. Dougan, M. Achtman, *Salmonella typhi*, the causative agent of typhoid fever, is approximately 50,000 years old, Infect. Genet. Evol. 2 (2002) 39–45.

[32] H. Zeng, K. De Reu, S. Gabriël, W. Mattheus, L. De Zutter, G. Rasschaert, *Salmonella* prevalence and persistence in industrialized poultry slaughterhouses, Poultry Sci. 100 (2021) 100991.

[33] V.M. Duc, Y. Nakamoto, A. Fujiwara, H. Toyofuku, T. Obi, T. Chuma, Prevalence of *Salmonella* in broiler chickens in Kagoshima, Japan in 2009 to 2012 and the relationship between serovars changing and antimicrobial resistance, BMC Vet. Res. 15 (2019) 1–8.

[34] E. Álvarez-Fernández, C. Alonso-Calleja, C. García-Fernández, R. Capita, Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from poultry in Spain: comparison between 1993 and 2006, Int. J. Food Microbiol. 153 (2012) 281–287.

[35] M.M. Elsayed, Y.F.H. El-Basrey, A.H. El-Baz, H.A. Dowidar, A. Shami, F.A. Al-Saeed, A. Alsamghan, H.M. Salem, W.A. Alhazmi, K.A. El-Tarabily, Ecological prevalence, genetic diversity, and multidrug resistance of *Salmonella* Enteritidis recovered from broiler and layer chicken farms, Poultry Sci. 103 (2024) 103320.

[36] G. Mead, A.M. Lammerding, N. Cox, M.P. Doyle, F. Humbert, A. Kulikovskiy, A. Panin, V.P. Do Nascimento, M. Wierup, General interest scientific and teechnical factors affecting the setting of *Salmonella* criteria for raw poultry: a global perspective, J. Food Protect. 73 (2010) 1566–1598.

[37] D.E. Cosby, N.A. Cox, M.A. Harrison, J.L. Wilson, R.J. Buhr, P.J. Fedorka-Cray, *Salmonella* and antimicrobial resistance in broilers: a review, J. Appl. Poultry Res. 24 (2015) 408–426.

[38] E.A. McMillan, J.L. Wasilenko, K.A. Tagg, J.C. Chen, M. Simmons, S.K. Gupta, G.E. Tillman, J. Folster, C.R. Jackson, J.G. Frye, Carriage and gene content variability of the pESI-like plasmid associated with *Salmonella* Infantis recently established in United States poultry production, Genes 11 (2020) 1516.

[39] L. Lapierre, J. Cornejo, S. Zavala, N. Galarce, F. Sánchez, M.B. Benavides, M. Guzmán, L. Sáenz, Phenotypic and genotypic characterization of virulence factors and susceptibility to antibiotics in *Salmonella* infantis strains isolated from chicken meat: first findings in Chile, Animals 10 (2020) 1049.

[40] F. Shahada, T. Chuma, G. Kosugi, M. Kusumoto, T. Iwata, M. Akiba, Distribution of extended-spectrum cephalosporin resistance determinants in *Salmonella* enterica andEscherichia coli isolated from broilers in southern Japan, Poult, Science 92 (2013) 1641–1649.

[41] E.P. Mendonça, R.T. Melo, M.R.M. Oliveira, G.P. Monteiro, P.A.B.M. Peres, B.B. Fonseca, A. Giombelli, D.A. Rossi, Characteristics of virulence, resistance and genetic diversity of strains of *Salmonella* Infantis isolated from broiler chicken in Brazil, Pesqui, Veterinária Bras. 40 (2020) 29–38.

[42] M.B. Kim, H.-R. Jung, Y.J. Lee, Emergence of *Salmonella* Infantis carrying the pESI megaplasmid in commercial farms of five major integrated broiler operations in Korea, Poultry Sci. (2024) 103516.

[43] A. Patrícia, N. Carla, P. Luísa, Food-to-humans bacterial transmission, Microbiol. Spectr. 8 (2020), https://doi.org/10.1128/microbiolspec.mtbp-0019–2016.

[44] H. Wu, Y. Wang, Y. Wu, J. Qiao, H. Li, S. Zheng, X. Xia, S. Cui, X. Wang, M. Xi, Emergence of β-lactamases and extended-spectrum β-lactamases (ESBLs) producing *Salmonella* in retail raw chicken in China, Foodborne Pathog, Dissent 12 (2015) 228–234.

[45] W.W.Y. Lee, J. Mattock, D.R. Greig, G.C. Langridge, D. Baker, S. Bloomfield, A.E. Mather, J.R. Wain, A.M. Edwards, H. Hartman, Characterization of a pESI-like plasmid and analysis of multidrug-resistant *Salmonella enterica* Infantis isolates in England and Wales, Microb. Genom. 7 (2021) 658.

[46] L. Mejía, J.L. Medina, R. Bayas, C.S. Salazar, F. Villavicencio, S. Zapata, J. Matheu, J.A. Wagenaar, F. González-Candelas, C. Vinueza-Burgos, Genomic epidemiology of *Salmonella* Infantis in Ecuador: from poultry farms to human infections, Front. Vet. Sci. 7 (2020) 547891.

[47] N. Davies, F. Jørgensen, C. Willis, J. McLauchlin, M.A. Chattaway, Whole genome sequencing reveals antimicrobial resistance determinants (AMR genes) of *Salmonella enterica* recovered from raw chicken and ready-to-eat leaves imported into England between 2014 and 2019, J. Appl. Microbiol. 133 (2022) 2569–2582.

[48] C. Garcia, N. Hinostroza, L. Astocondor, T. Ochoa, J. Jacobs, Characterization of ESBL-producing *Salmonella enterica* serovar infantis infection in humans, Lima, Peru, Am. J. Trop. Med. Hyg. 101 (2019) 746.

[49] M.E. Srednik, B.R. Morningstar-Shaw, J.A. Hicks, C. Tong, T.A. Mackie, L.K. Schlater, Whole-genome sequencing and phylogenetic analysis capture the emergence of a multi-drug resistant *Salmonella enterica* serovar Infantis clone from diagnostic animal samples in the United States, Front. Microbiol. 14 (2023) 1166908.

[50] W. Wang, L. Zhao, Y. Hu, T. Dottorini, S. Fanning, J. Xu, F. Li, Epidemiological study on prevalence, serovar diversity, multidrug resistance, and CTX-M-type extended-spectrum β-lactamases of *Salmonella* spp. from patients with diarrhea, food of animal origin, and pets in several provinces of China, Antimicrob. Agents Chemother. 64 (2020) 10–1128.

[51] S. Petrin, M. Orsini, E. Mastrorilli, A. Longo, D. Cozza, J.E. Olsen, A. Ricci, C. Losasso, L. Barco, Identification and characterization of a spreadable IncI1 plasmid harbouring a *bla*$_{CTX-M-15}$ gene in an Italian human isolate of *Salmonella* serovar Napoli, Plasmid 114 (2021) 102566.

[52] A.F. Mechesso, S.-H. Na, D.C. Moon, S.-J. Kim, J.-H. Choi, H.-J. Song, S.-S. Yoon, S.-K. Lim, Antibiotic resistance profiles and molecular characteristics of *bla*$_{CTX-M-15}$-carrying *Salmonella enterica* serovar Enteritidis isolates from healthy and diseased chickens in Korea, Foodb. Pathog. Dis. 19 (2022) 663–674.

[53] Q. Moura, M.R. Fernandes, K.C. Silva, D.F. Monte, F. Esposito, M. Dropa, C. Noronha, A.M. Moreno, M. Landgraf, F.J. Negrão, N. Lincopan, Virulent nontyphoidal *Salmonella* producing CTX-M and CMY-2 β-lactamases from livestock, food and human infection, Brazil, Virulence 9 (2018) 281–286.

[54] S. Saito, Y. Koori, Y. Ohsaki, S. Osaka, K. Oana, Y. Nagano, Y. Arakawa, N. Nagano, Third-generation cephalosporin-resistant non-typhoidal *Salmonella* isolated from human feces in Japan, Jpn. J. Infect. Dis. 70 (2017) 301–304.
[55] M.S. Diab, A.S. Thabet, M.A. Elsalam, R.M. Ewida, S.A. Sotohy, Detection of Virulence and β-lactamase resistance genes of non-typhoidal *Salmonella* isolates from human and animal origin in Egypt, Gut Pathog. 15 (2023) 1–9.
[56] E. Liebana, A. Carattoli, T.M. Coque, H. Hasman, A.-P. Magiorakos, D. Mevius, L. Peixe, L. Poirel, G. Schuepbach-Regula, K. Torneke, Public health risks of enterobacterial isolates producing extended-spectrum β-lactamases or AmpC β-lactamases in food and food-producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control options, Clin. Infect. Dis. 56 (2013) 1030–1037.
[57] C. Dierikx, A. van Essen-Zandbergen, K. Veldman, H. Smith, D. Mevius, Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry, Vet. Microbiol. 145 (2010) 273–278.
[58] B. Papić, D. Kušar, J. Mićunović, M. Pirš, M. Ocepek, J. Avberšek, Clonal Spread of pESI-positive multidrug-resistant ST32 *Salmonella enterica* serovar infantis isolates among broilers and humans in Slovenia, Microbiol. Spectr. 10 (2022).
[59] W. Wang, J. Cui, Y. Hu, F. Li, X. Deng, Y. Dong, S. Li, Genomic characterization of *Salmonella* isolated from retail chicken and humans with diarrhea in Qingdao, China, Front. Microbiol. 14 (2023) 1295769.
[60] J.-H. Park, H.-S. Kim, J.-H. Yim, Y.-J. Kim, D.-H. Kim, J.-W. Chon, H. Kim, A.-S. Om, K.-H. Seo, Comparison of the isolation rates and characteristics of *Salmonella* isolated from antibiotic-free and conventional chicken meat samples, Poultry Sci. 96 (2017) 2831–2838.