Conjugative Plasmid-Mediated Extended Spectrum Cephalosporin Resistance in Genetically Diverse *Escherichia coli* from a Chicken Slaughterhouse

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**Simple Summary:** Extended-spectrum cephalosporin (ESC)-resistant *Enterobacteriaceae* frequently detected in humans and food-producing animals are of major concern in public health. This study was undertaken to investigate the contamination of ESC-resistant *E. coli* in the environment of a slaughterhouse during chicken meat processing. This study indicates that cross-contamination of ESC/ AmpC-producing *E. coli* has a crucial impact on the occurrence of ESC resistance in retail chicken meat. Thus, ESBL-/ AmpC-producing *E. coli* were brought into the slaughterhouse by certain broiler chicken flocks, and other chicken flocks were contaminated by ESBL/AmpC-producing *E. coli* already present in the slaughterhouse environment. These findings support the hypothesis that ESC resistance in broiler chickens in Korea. As conjugative plasmids always carrying multiple resistance genes, continuous persistence of *bla*CTX-M and *bla*CMY genes located on plasmids within microbial communities will be mediated by co-selection processes with other resistance genes. Hence, further research on the control of bacterial conjugation is urgently required. Our study emphasizes that chicken slaughterhouses could perform the functions of convergence and dispersion of ESBL/AmpC resistance, and that world widely epidemic conjugative plasmids contribute to the dissemination of ESBL/AmpC from chickens to humans along the food chain.

**Abstract:** ESC-resistant *E. coli* isolates were collected from broiler chickens, a slaughterhouse, and retail meat to assess their dispersion and their involvement in cross-contamination. ESBL-/AmpC-producing *E. coli* were isolated during the slaughter process of all six investigated chicken flocks from scalding, feather removal, first conveyor, evisceration, second washing, third conveyor, and third washing areas, and from handling workers in the slaughterhouse. ESC-resistant *E. coli* isolates with the same pulsed-field gel electrophoresis type were found in the same site (scalding) on different sampling days. ESBL/AmpC-producing *E. coli* isolates were absent in the lairage area in the slaughterhouse, but present in the retail markets in 36.8% (7/19) of the chicken flocks. The *bla*CTX-M genes and *bla*CMY-2 were conjugated to recipient *E. coli* J53 in 67.5% (27/40) and 56.1% (23/41) of ESBL-producing and AmpC-producing *E. coli* isolates, respectively. The presence of the same conjugative plasmids was found in genetic diversity ESC-resistant *E. coli* colonies collected on different sampling days. Our study emphasizes that cross-contamination of ESBL/AmpC-producing *E. coli* in slaughterhouse has a crucial impact on the occurrence of ESC resistance in retail chicken meat.

**Keywords:** extended-spectrum cephalosporins; *E. coli*; chicken; slaughterhouse; genetically diverse; conjugative plasmid
1. Introduction

Bacterial resistance, especially to 3rd generation cephalosporins, is of great concern to public health due to the limitations of choice of therapy for human infections. Extended-spectrum cephalosporin (ESC)-resistant *Enterobacteriaceae* are frequently detected in humans and food-producing animals, as well as in the environment [1]. As food-producing animals, especially broiler chickens, are considered possible reservoirs for ESC-resistant *Enterobacteriaceae*, meat and other foodstuffs of animal origin are considered potential sources for the colonization or infection of humans [2].

*Escherichia coli* isolates resistant to ESC mainly due to the acquisition of the resistant genes encoding for extended-spectrum β-lactamas (ESBLs) and plasmid-mediated AmpC (pAmpC) enzymes. The ESBLs consist of several families, and the main enzymes in ESC-resistant *E. coli* are the CTX-M and SHV types, and CMY-type enzymes are the most frequently reported pAmpC β-lactamase [3,4]. Conjugative plasmids carrying ESBL and pAmpC genes are frequently co-harbored genes encoding resistant to other antibiotics, and these conjugative plasmids could maintain and transfer within different bacterial communities. The plasmids harbored ESBL and pAmpC genes have been associated with different transferable replicon types, such as IncA/C and IncI1 [3–5]. *E. coli* strains bearing plasmids with ESC resistance, capable of successful conjugative transfer in chicken, could promote the horizontal spread and dissemination to other bacterial hosts, from food-producing animals to humans [6]. This may also play a crucial role in the spread and maintenance of ESC resistance in broiler chicken production.

It has been suggested that there are many stages during the poultry slaughtering process where cross-contamination of foodborne pathogens of *Salmonella* and *Campylobacter* can occur [7,8]. There is, however, only limited evidence available that demonstrates the cross-contamination of ESC-resistant *E. coli* in slaughterhouses [9]. Research has shown that ESC-resistant *E. coli* transmission may occur throughout the whole poultry production chain, and the possible contamination of chicken carcasses with ESC-resistant *E. coli* within the chicken slaughterhouse. This study was undertaken to investigate the ESC-resistant *E. coli* contamination in the slaughterhouse environment along the chicken processing chain. ESC-resistant *E. coli* isolates from broiler chickens, the slaughterhouse environment, and retail meat products, were investigated in order to (i) define the clonal relationships between the isolates for the assessment of the dissemination of the recovered ESC-resistant strains and their involvement in cross-contamination, and (ii) assess the horizontal ESC resistant gene transfer in *E. coli* isolates from broiler chicken slaughterhouses and retail meat by conjugation assays.

2. Materials and Methods

2.1. Sampling

Twenty-five Korean broiler chicken flocks were investigated for ESC-resistant *E. coli* from November 2015 to October 2016. All chicken flocks were slaughtered at the same slaughterhouse, which is the largest chicken slaughter and processing plant in Korea. The size of the flocks ranged from 50,000 to 100,000 broiler chickens. All 25 flocks were sampled on separate days.

The sampling was performed as follows: firstly, fresh pooled samples of chicken feces were collected from all over the lairage area, and 3–6 pooled samples were collected from each broiler chicken flock. The environmental samples were collected from the first batch on each sampling day at 6 time points from 12 slaughterhouse process sites, including (1) lairage, (2) scalding, (3) feather removal, (4) the first conveyor, (5) evisceration, (6) the first washing, (7) the second conveyor, (8) the second washing, (9) air chilling, (10) the third conveyor, (11) the third washing, and (12) handling workers along the entire poultry processing operation in the slaughterhouse. All samples were collected using sterile cotton gauze, as described previously [8]. All samples were transported to the laboratory on the same day in cooled transport containers with ice for immediate analysis.
Feces samples were collected from an additional 19 broiler chicken flocks from the lairage area and downstream retail chicken meat from the same batch of broiler chicken in the retail market. The raw whole-chicken samples (12–15 samples per flock) were purchased from the retail supermarket and placed on ice in cooled containers and returned to the laboratory for processing within 24 h.

2.2. Isolation and Detection

All samples were to investigate the presence of ESC-resistant *E. coli* by selective plating on MacConkey (MC; BD Difco, Sparks, MD, USA) agar supplemented with ceftiofur (8 µg/mL). All colonies on MacConkey agar with different color and morphology were picked and sub-cultured on blood agar plates (Komed, Seongnam, Korea) and confirmed by polymerase chain reaction (PCR) [10]. Three to five typical colonies were collected for each sample. The minimum inhibitory concentrations (MICs) of 16 antimicrobials were determined for confirmed *E. coli* isolates by using the KRNV5F Sensititre™ Broth Microdilution System panel (TREK Diagnostic Systems, Incheon, Korea).

2.3. Molecular Characterization

Multiplex PCRs were performed to confirm ESBL/pAmpC resistance genes, and DNA sequence of the resistant genes was performed using an ABI3710 automated sequencer (SolGent, Daejeon, Korea), and sequence comparisons are performed with BLAST (Basic Local Alignment Search Tool). Pulsed-field gel electrophoresis (PFGE) was performed with *XbaI* for all the *E. coli* pAmpC-producing isolates, as described previously (https://pulsenetinternational.org, accessed on 20 August 2018).

2.4. Conjugation Assay and Molecular Characterization of the Transconjugants

Transfer of ESBL/pAmpC genes to the sodium azide resistant *E. coli* J53 by conjugation was determined by broth-mating experiments [11]. MacConkey agar containing 100 µg/L of sodium azide and 4 µg/mL of ceftiofur was used to select the transconjugants. All the transconjugants were to determine the MICs and the presence of ESBL/pAmpC genes, as described above. Plasmid DNA was extracted from the transconjugants culture using a plasmid Miniprep kit according to the manufacturer’s instructions (Life Technologies, Waltham, MA, USA). The extracted plasmids were analyzed by PCR-based replicon typing [12]. The IncI1 plasmids were further characterized by plasmid multi-locus sequence typing (pMLST) (https://pubmlst.org/plasmid/, accessed on 5 November 2020).

3. Results

3.1. Distribution of ESBL/AmpC Genes in *E. coli* Isolates from the Slaughterhouse and Retail Meat

In total, 81 suspected ESBL/AmpC-producing *E. coli* isolates were collected, and all isolates were used to examine the presence of the β-lactamase encoding genes, *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>TEM</sub>, and the AmpC β-lactamase gene, *bla*<sub>CMY</sub>. Among these 81 isolates, the presence of *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, and *bla*<sub>CMY</sub> was confirmed in 78 isolates (Table 1). The dominant CTX-M types included *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-14</sub>, while *bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-55</sub> and *bla*<sub>CTX-M-65</sub> were detected in ESBL-producing isolates. Moreover, the only AmpC gene, *bla*<sub>CMY-2</sub>, was found both in the slaughterhouse and retail chicken meat.

Transfer of *bla*<sub>CTX-M</sub> genes to recipient *E. coli* J53 were found in 67.5% (27/40) of the ESBL-producing isolates. Three *bla*<sub>CTX-M</sub> genes (*bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-14</sub>, and *bla*<sub>CTX-M-55</sub>) were transferred to J53 in the ESBL-producing *E. coli* isolates from the lairage area, the slaughterhouse environment, and retail meat. The AmpC gene, *bla*<sub>CMY-2</sub>, could be transferred to J53 in 56.1% (23/41) of the AmpC-producing *E. coli* isolates, and its presence was confirmed in AmpC-producing isolates from the lairage area, the slaughterhouse environment, and retail meat.
Table 1. ESC resistance genes in ESC- *E. coli* isolated from the slaughterhouse and retail meat.

| Phenotype | Resistance Genes | No. of Isolates | Conjugation Result (No.) |
|-----------|-----------------|----------------|-------------------------|
|           |                 | Slaughterhouse | Retail Meat | Slaughterhouse | Retail Meat |
|           |                 | Lairage | Slaughterhouse | Environment | Lairage | Slaughterhouse | Environment |
| ESBL      | NA              | 3       | 3             |             |             |             |
|           | TEM-1           | 5       | 5             |             |             |             |
|           | CTX-M-1         | 6       | 5             | 1           | CTX-M-1     | 6       | 5             | 1           |
|           | CTX-M-14        | 7       | 6             | 1           | CTX-M-14    | 7       | 6             | 1           |
|           | CTX-M-15        | 2       | 2             |             |             |             |
|           | CTX-M-55        | 2       | 1             | 1           |             |             |
|           | CTX-M-65        | 1       | 1             |             |             |             |
|           | TEM-1, CTX-M-1  | 8       | 4             | 3           | 1           | CTX-M-1     | 8       | 4             | 3           | 1           |
|           | TEM-1, CTX-M-14 | 2       | 2             | 2           | CTX-M-14    | 2       | 2             | 2           |
|           | TEM-1, CTX-M-55 | 4       | 2             | 2           | TEM-1, CTX-M-55 | 4       | 2             | 2           |
| AmpC      | CMY-2           | 24      | 8             | 12          | 4           | CMY-2     | 14      | 4             | 8           | 2           |
|           | TEM-1, CMY-2    | 16      | 12            | 4           | CMY-2     | 8       | 6             | 2           |
|           | TEM-135, CMY-2  | 1       | 1             | 1           | CMY-2     | 1       | 1             |             |

NA, not available.
3.2. Occurrence of ESBL/AmpC genes in E. coli Isolates in the Slaughterhouse Environment

The presence of ESBL/pAmpC genes in the E. coli isolates in the environment was confirmed during 6 visits on different sampling days to the same slaughterhouse (Table 2). The ESBL/pAmpC genes in E. coli were isolated from the scalding, feather removal, first conveyor, evisceration, second washing, third conveyor, and third washing stages, as well as from the handling workers in the slaughterhouse. ESC-resistant E. coli were not detected in the first washing, the second conveyor, and air chilling sites in the slaughterhouse.

The clonality of the ESC-resistant E. coli isolates from the slaughterhouse typed by XbaI-PFGE is shown in Table 2 and Supplementary Figure S1. Clonal diversity of the ESC-resistant E. coli isolates was found within and between different sampling days (flocks 1 to 6) in the slaughterhouse environment. However, ESC-resistant E. coli isolates with the same PFGE type (type 54) were found in the same site (the scalding area) in the slaughterhouse on different sampling days (flocks 3 and 4).

The conjugative plasmids in ESC-resistant E. coli isolates from the slaughterhouse are shown in Table 2. Five types of conjugative plasmids (blaCTX-M-1 IncI1/ST87,blaTEM-1,CTX-M-55 IncFIB, blaCMY-2 IncI1-ST12, blaCMY-2 IncI1-ST18, and blaCMY-2 IncI1-ST86) were found in ESC-resistant E. coli isolates from the slaughterhouse environment. A comparison of the high genetic diversity of the ESC-resistant E. coli colonies from the slaughterhouse environment revealed the presence of the same conjugative plasmids in different ESC-resistant E. coli colonies collected on different sampling days.

Of particular interest was the finding that the ESC-resistant E. coli isolates from the scalding area of sampling no. 3 and 4 with the same PFGE type 54 contained different conjugative plasmids—blaCMY-2 IncI1-ST18 and blaCMY-2 IncI1-ST12. This plasmid, blaCMY-2 IncI1-ST12, was found in the feather removal and evisceration sites in the slaughterhouse for sampling no. 4. Although ESC-resistant E. coli isolates with blaCTX-M-1 IncI1/ST87 were isolated from the slaughterhouse environment (sampling no. 5, from the feather removal and third conveyor sites), the corresponding flock in the lairage area was negative for ESC-resistant E. coli.

3.3. Correlation between ESBL/AmpC-Producing E. coli from the Slaughterhouse and Retail Meat

Among the 19 chicken flocks (flocks 7–25) sampled in this study, ESBL/AmpC-producing E. coli was detected in 36.8% (7/19) of the chicken flocks (flock 8, 10, 12, 14, 17, 21, and 22). These isolates were found in chicken from the retail markets, but not from the lairage area in the slaughterhouse. Further, 31.6% (6/19) of the ESBL/AmpC-producing E. coli-positive chicken flocks did not show the presence of these isolates after processing in the slaughterhouse.

All ESBL/AmpC-producing E. coli isolates from the lairage area in the slaughterhouse and the downstream retail meat from the retail market were further analyzed by XbaI-PFGE typing and conjugative plasmid typing (Table 3). Most of the ESBL/AmpC-producing E. coli isolates from different chicken flocks from the slaughterhouse and retail market showed genetic diversity of PFGE types. E. coli isolates with PFGE type 58 were found in different flocks from the lairage area in the slaughterhouse (flock 7) and retail meat (flock 14). E. coli isolates from the lairage area in the slaughterhouse with identified PFGE types 10 and 31 were also found in different flocks, namely 9 and 23, and 18 and 20, respectively. Comparing the PFGE genetic diversity of the E. coli isolates from the slaughterhouse and retail meat led to the identification of the conjugative plasmids, blaCTX-M-14 IncI1/ST38, blaCTX-M-14 IncI1/ST87, and blaCMY-2 IncI1-ST12, in E. coli isolates with different PFGE types from the lairage area and retail meat. Two conjugative plasmids, blaCTX-M-1 IncI1/ST87 and blaTEM-1,CTX-M-55 IncN, isolated from retail meat were not found in the lairage area in the slaughterhouse.
Table 2. PFGE types and conjugative plasmid types of ESC-E. coli isolates along the slaughter-line in the slaughterhouse.

| Flocks No. (Date) | Typing | Slaughterhouse Processing * |
|-------------------|--------|-----------------------------|
|                   |        | 1. Lairage | 2. Scalding | 3. Feather Removal | 4. First-Convey | 5. Evisceration | 6. Second-Washing | 7. 8. | 9. | 10. | 11. | 12. Handling Workers |
| 1 (160405)        | PFGE type | 11, 20, 29, 37, 49, 56 | 9 | 1, 22 | 22 | NA | NA | NA | NA | NA | NA | NA | 52 |
|                   | Conjugative plasmids | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | |
| 2 (160407)        | PFGE type | 23, 30, 45, 60, 63 | 2 | NA | NA | NA | NA | NA | NA | NA | NA | NA | |
|                   | Conjugative plasmids | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | |
| 3 (160412)        | PFGE type | 5, 17 | 54 | 40 | 27 | NA | NA | NA | NA | NA | NA | NA | |
|                   | Conjugative plasmids | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | |
| 4 (160414)        | PFGE type | 6, 19, 35, 48, 51 | 54 | 54 | 54 | NA | NA | NA | NA | NA | NA | NA | |
|                   | Conjugative plasmids | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | |
| 5 (160419)        | PFGE type | 32 | 32 | 25 | NA | NA | NA | NA | NA | NA | NA | NA | |
|                   | Conjugative plasmids | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | |
| 6 (160428)        | PFGE type | 13, 16 | 21, 22 | 33 | NA | NA | NA | NA | NA | NA | NA | NA | |
|                   | Conjugative plasmids | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | |

* The processing of 1 to 12 represents lairage (1), scalding (2), feather removal (3), the first conveyor (4), evisceration (5), the first washing (6), the second conveyor (7), the second washing (8), air chilling (9), the third conveyor (10), the third washing (11), and workers (12). Blank means no isolates; NA means not transferable. ESC-resistant E. coli were not detected in the first washing (6), the second conveyor (7), and air chilling (9) in the slaughterhouse.
Table 3. PFGE types and conjugative plasmid types of ESC- *E. coli* isolates from slaughterhouse and retail market.

| Flock No. | Lairage \(^a\) | **Slaughter-Line** | **Retail Meat** |
|----------|----------------|-------------------|-----------------|
|          | PFGE Type | Conjugative Plasmid | PFGE Type | Conjugative Plasmid | PFGE Type | Conjugative Plasmid |
| 1        | 11, 20, 29, 37, 49, 56 | NA | 1, 9, 22, 52 | bla\(_{\text{CMY-2}}\) | IncI1-ST86 | nd |
|          |           |                   |            | bla\(_{\text{CMY-2}}\) | IncI1-ST12 | nd |
| 2        | 23, 30, 45, 60, 63 | NA, bla\(_{\text{CTX-M-1}}\) | IncI1-ST87 | 2 | bla\(_{\text{CMY-2}}\) | IncI1-ST12 | nd |
| 3        | 5, 17 | bla\(_{\text{CTX-M-1}}\) | IncI1-ST87 | 27, 40, 54 | bla\(_{\text{TEM-1,CTX-M-55}}\) | IncFIB | nd |
|          |           |                   |            | bla\(_{\text{CTX-M-1}}\) | IncI1-ST87 | nd |
| 4        | 6, 19, 35, 48, 51 | bla\(_{\text{CMY-2}}\) | IncI1-ST12 | 54 | bla\(_{\text{CMY-2}}\) | IncI1-ST12 | nd |
| 5        | - | - | 25, 32 | bla\(_{\text{CTX-M-1}}\) | IncI1-ST87 | nd |
| 6        | 13, 16 | bla\(_{\text{CMY-2}}\) | IncI1-ST108 | 21, 22, 33 | bla\(_{\text{CMY-2}}\) | IncI1-ST12 | nd |
|          |           |                   |            | bla\(_{\text{TEM-1,CTX-M-55}}\) | IncFIB | nd |
| 7        | 38, 53, 58, 59, 62 | bla\(_{\text{CMY-2}}\) | IncI1-ST12 | nd | - | - |
| 8        | - | - | nd | 4 | bla\(_{\text{CTX-M-1}}\) | IncI1-ST87 |
| 9        | 8, 10, 42 | bla\(_{\text{CTX-M-14}}\) | IncI1-ST162 | nd | - | - |
| 10       | - | - | nd | 36 | bla\(_{\text{TEM-1,CTX-M-55}}\) | IncN |
| 11       | 7, 41, 55 | NA | nd | nd | nd | nd |
| 12       | - | - | nd | 34 | bla\(_{\text{TEM-1,CTX-M-55}}\) | IncN |
| 13       | nd | nd | nd | 43 | bla\(_{\text{CTX-M-1}}\) | IncI1-ST87 |
| 14       | - | - | nd | 58 | bla\(_{\text{CMY-2}}\) | IncI1-ST12 |
| 15       | 3, 15 | bla\(_{\text{CTX-M-14}}\) | IncI1-ST38 | nd | 15 | bla\(_{\text{CMY-2}}\) | IncI1-ST12 |
| 16       | nd | nd | nd | 62 | NA |
| 17       | - | - | nd | 50 | bla\(_{\text{CTX-M-14}}\) | IncI1-ST87 |
| 18       | 31, 47 | bla\(_{\text{CMY-2}}\) | IncI1-ST86 | nd | - | - |
| 19       | 26, 57 | NA | nd | 22, 24 | NA |
| 20       | 18, 31 | bla\(_{\text{CTX-M-14}}\) | IncI1-ST162 | nd | - | - |
| 21       | - | - | nd | 61 | bla\(_{\text{CTX-M-14}}\) | IncI1-ST162 |
### Table 3. Cont.

| Flock No. | Lairage | Slaughter-Line | Retail Meat |
|-----------|---------|----------------|-------------|
|           | PFGE Type | Conjugative Plasmid | PFGE Type | Conjugative Plasmid | PFGE Type | Conjugative Plasmid |
| 22        | -       | - | nd | 25 | bla CMY-2 IncI1-ST112 |
| 23        | 10, 12, 39 | NA, bla CTX-M-1 IncI1-ST38 | nd | 44 | bla CTX-M-14 IncI1-ST38 |
| 24        | 28       | bla CTX-M-14 IncI1-ST12 | nd | - | - |
| 25        | 14, 46   | bla CTX-M-14 IncI1-ST187 | nd | - | - |

- means no ESC-resistant isolates were obtained from the MacConkey agar with ceftiofur; nd means not done; and NA means not transferable.

### 4. Discussion

Antibiotic resistance is an increasing and evolving phenomenon, with infections due to ESBL/AmpC-producing bacteria being associated with significant morbidity and mortality worldwide [13]. Poultry are the main animal species involved in the spread of these pathogens, and the occurrence of such resistant bacteria in poultry indicates that they may serve as reservoirs of ESC resistance genes, which disseminate to other bacterial species along the food chain [14]. In the present study, the occurrence and distribution of ESBL/AmpC-producing E. coli in a slaughterhouse and its downstream retail meat in Korea were investigated. ESBL/AmpC-producing E. coli were widely found at various processing stages of chicken production, and certain transmission routes could be confirmed for some foodborne pathogens in chicken during processing in the slaughterhouse [7,8,15]. The environment in fattening farms for broiler chicken can be a source of ESBL/AmpC-producing E. coli contamination of retail meat [16]. The detection of ESBL/AmpC-producing E. coli in the slaughterhouse environment shows poor control measures for ESBL/AmpC-producing E. coli taken during the slaughtering processing, while most hygiene standards and control measures are based on indicative E. coli [17].

Cross-contamination in broiler chicken slaughterhouses between flocks is well known [18]. An insufficiently cleaned and disinfected environment may also be a source of poultry flock contamination during processing in the slaughterhouse. The persistence of some isolates in the slaughterhouse environment may constantly contaminate the chicken flocks handled subsequently [8]. In our results, the repeated recovery of ESBL/AmpC-producing E. coli isolates with PFGE type 54 from the scalding area in the slaughterhouse on different sampling days showed the potential contamination in the successive chicken flocks by the slaughterhouse environment. Furthermore, some isolates may form biofilms due to their long-term presence in the slaughterhouse environment and cause continuous contamination. This was confirmed by the recovery of the ESBL/AmpC-producing E. coli isolate with PFGE type 2 from the third conveyor area in the slaughterhouse (flock 5) and the subsequent recovery from the retail meat of flock no. 22, which was originally ESBL/AmpC-producing E. coli-negative. Therefore, in this scenario, the chicken slaughterhouse performed the functions of convergence and dispersion, with various ESBL/AmpC-producing E. coli from chicken flocks from different regions being gathered in the same slaughterhouse and dispersed downstream to the retail meat markets located in different regions. In addition, the ESBL/AmpC-producing E. coli-positive retail meat from ESBL/AmpC-producing E. coli-negative chicken flocks (36.8%, 7/19) in this study emphasizes the role of the slaughterhouse in gathering and spreading the ESBL/AmpC-producing E. coli isolates. Furthermore, knowledge of these contamination sources and dissemination modes should be harnessed for the development and application of effective intervention measures against the dissemination of ESBL/AmpC producers during processing in the slaughterhouse.

In addition, slaughterhouses may offer sites for further transfer and dissemination of ESBL/AmpC-resistant genes between various bacterial strains. As shown in our results...
(Table 2), ESBL/AmpC-producing *E. coli* isolates with PFGE type 54 harboring the conjugative plasmid, *bla*\textsubscript{CMY-2} IncI1-ST18, were first (sampling date 160412) recovered from the scalding area in the slaughterhouse, while the isolates of PFGE type 54 harboring *bla*\textsubscript{CMY-2} IncI1-ST12 were recovered from the subsequent sampling at the scalding, feather removal, and evisceration sites. This result suggests the ability of the persistent *E. coli* flora of PFGE type 54 in the slaughterhouse to gain different conjugative plasmids. Studies have confirmed the highly efficient horizontal transfer of resistant plasmids by conjugation in bacterial biofilms and biofilm synthesis by ESBL-producing *E. coli* on numerous surfaces in the environment, including the food chain [19–21]. The higher frequency of transfer of the ESBL/AmpC-resistant plasmids in biofilms and the concurrent presence of other antibiotic resistance genes in these plasmids may have serious implications for public health [21]. Thus, it is essential to understand horizontal gene transfer in biofilms and how surface properties in slaughterhouses affect plasmid conjugation, which will aid in controlling resistant plasmid transfer in the future.

Conjugation is the most common way of transferring genetic information and plays a very important role in the spread of multiple antibiotic resistance genes [22]. In this study, 61.7% (50/81) of the ESBL/AmpC-producing *E. coli* isolates showed successful transfer of their ESBL/AmpC resistance by conjugation to another *E. coli* strain. These results suggest that under certain selective pressures, the resistant plasmids were very easily transferred between *E. coli* strains, leading to the spread of ESBL/AmpC resistance, which can be extremely harmful in clinical settings [23]. At several levels of the broiler chicken production chain, *E. coli* isolates harboring conjugative plasmids with *bla*\textsubscript{CMY} and *bla*\textsubscript{CTX-M} genes may facilitate horizontal spread and dissemination to other bacterial species in the environment [24]. In addition, *E. coli* strains with conjugative plasmids that persist in the chicken slaughterhouse environment may play an important role in the transmission and maintenance of ESC resistance in the broiler chain, environment, and clinical settings.

Although the IncI1 plasmids—the main type of plasmids found in this study—belong to the narrow-host-range type, IncI1 could successfully spread between different various *Enterobacteriaceae* species and other bacteria in the chicken meat production environment [24]. A noteworthy finding in the present study was that these conjugative plasmids carrying ESBL/AmpC resistance are frequently identified in human clinical isolates, highlighting the potential transfer of these resistant plasmids to humans through the food chain. The most commonly reported AmpC β-lactamase is CMY-2, and plasmid AmpC beta-lactamase CMY-2 is distributed worldwide, particularly in *E. coli* and *Salmonella* from food-producing animals and humans [3]. In this study, all the conjugative CMY-2 genes were located on the IncI1 plasmid, which has become one of the most common plasmid families worldwide [25]. The *bla*\textsubscript{CMY-2} IncI1—belonging to four different STs (ST12, ST18, ST86, and ST108) in our results and the pMLST database—were primarily isolated from *E. coli* and *Salmonella* from humans, poultry, and swine in the USA, Canada, UK, and Korea [25]. Notably, the plasmid *bla*\textsubscript{CMY-2} IncI1-ST12 has been described in several continents, with hosts consisting of both zoonotic pathogens and commensal bacterial species, circulating in both humans and food-producing animals [26–28]. This type of plasmid exhibits all the characteristics of a worldwide epidemic plasmid, and the widespread presence of *bla*\textsubscript{CMY-2} IncI1-ST12 in the chicken meat production chain and the slaughterhouse environment suggests its epidemic potential in Korea in the future [29]. Although the plasmid *bla*\textsubscript{CMY-2} IncI1 has not been widely reported in ST18, ST86, and ST108, the identification of these conjugative plasmids in clonally diverse *E. coli* isolates may imply its successful dissemination in the chicken meat production chain and retail products in Korea. Therefore, continual surveillance of these plasmids in the broiler chicken meat production chain is essential to reveal the extent of its threat to public health.

IncI1 is recognized as one of the most pervasive plasmids in ESC resistant bacteria found in foods of animal origin [25,30]. Moreover, with *bla*\textsubscript{CTX-M-1} and *bla*\textsubscript{CTX-M-14} also being commonly found worldwide, the association between the CTX-M and the IncI1 type of plasmid has been described extensively [24,25,31]. In this study, *bla*\textsubscript{CTX-M-14}-carrying
plasmids predominantly belonged to the IncI1 type. These results are contradictory to the findings of a previous study in Korea, which reported the spread of \( \text{bla}_{\text{CTX-M-14}} \) in \( E. \text{coli} \) to be mediated mainly by IncF plasmids [32]. It is important to note that \( \text{bla}_{\text{CTX-M-14}} \) located on the IncI1 plasmid might have some evolutionary advantage, as it is the only \( \text{bla}_{\text{CTX-M}} \) located on the IncI1 plasmid identified in this study. In addition, we found that \( \text{bla}_{\text{CTX-M-14}} \) located on the plasmid IncI1 belonged to ST38 and ST87 in our isolates. IncI1 plasmids harboring \( \text{bla}_{\text{CTX-M-1}} \) \( E. \text{coli} \), commonly found in European broiler production, are found in \( E. \text{coli} \) isolates with genetic diversity, indicating that these plasmids successfully disseminate horizontally in the \( E. \text{coli} \) communities spreading from Korean broiler chicken as a source [25]. Our study demonstrates that, due to the dynamic evolution of ESC resistance and horizontal gene transfer of plasmids in food-producing animals, improving the surveillance of ESC resistance would be a key intervention in addressing the rise of resistant bacteria.

5. Conclusions

In summary, our results indicate that cross-contamination has a crucial role in the occurrence of ESBL/AmpC-producing \( E. \text{coli} \) in retail chicken meat. It was found that ESBL-/AmpC-producing \( E. \text{coli} \) were brought into the slaughterhouse by certain broiler chicken flocks, and other chicken flocks were contaminated by ESBL/AmpC-producing \( E. \text{coli} \) already present in the slaughterhouse environment. Our study also shows the need not only for intervention measures to prevent contamination with ESBL/AmpC-producing \( E. \text{coli} \) on the farm level, but also for effective interventions against cross-contamination with ESBL/AmpC-producing \( E. \text{coli} \) in the slaughterhouse. Therefore, improved cleaning and disinfection measures in the slaughterhouse should be emphasized to avoid further spread of ESBL/AmpC-producing \( E. \text{coli} \). Our results also support the hypothesis that these world widely epidemic conjugative plasmids have contributed to the dissemination of ESBL/AmpC resistance in broiler chickens in Korea. As conjugative plasmids always carry multiple resistance genes, future persistence of \( \text{bla}_{\text{CTX-M}} \) and \( \text{bla}_{\text{CMY}} \) genes within the bacterial population will be mediated by co-selection processes with other antimicrobial resistance genes. Hence, further research on the control of bacterial conjugation is urgently required.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ani11092491/s1, Figure S1. A dendrogram of PFGE profiles for 81 ESBL-/AmpC-producing \( E. \text{coli} \) isolates from the chicken production chain. AMR, antimicrobial resistance; ESC, extended-spectrum cephalosporin; SL, slaughterhouse; Me, retail meat; AMP, ampicillin; AMC, amoxicillin/clavulanic acid; FOX, cefoxitin; FEP, cefepime; TAZ, ceftazidime; XNL, ceftriaxone; GEN, gentamicin; STR, streptomycin; TET, tetracycline; CIP, ciprofloxacin; NAL, nalidixic acid; SXT, trimethoprim/sulfamethoxazole; FIS, sulfisoxazole; CHL, chloramphenicol; NA, not available; ST, sequence type.

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Informed Consent Statement: Not applicable (This study was not involving humans).

Data Availability Statement: The data presented in this study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. European Food Safety Authority (EFSA). The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. EFSA J. 2020, 18, e06007. [CrossRef]

2. Seiffert, S.N.; Hilty, M.; Perreten, V.; Endimiani, A. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: An emerging problem for human health? Drug Resist. Update 2013, 16, 22–45. [CrossRef] [PubMed]

3. Jacoby, G.A. AmpC beta-lactamases. Clin. Microbiol. Rev. 2009, 22, 161–182. [CrossRef]

4. Pitout, J.D.; Laupland, K.B. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: An emerging public-health concern. Lancet Infect. Dis. 2008, 8, 159–166. [CrossRef]

5. Hopkins, K.L.; Liebana, E.; Villa, L.; Batchelor, M.; Threlfall, E.J.; Carattoli, A. Replicon typing of Plasmids carrying CTX-M or CMY beta-lactamases circulating among Salmonella and Escherichia coli isolates. Antimicrob. Agents Chemother. 2006, 50, 3203–3206. [CrossRef] [PubMed]

6. Lazarus, B.; Paterson, D.L.; Mollinger, J.L.; Rogers, B.A. Do human extraintestinal Escherichia coli infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. Clin. Infect. Dis. 2015, 60, 439–452. [CrossRef] [PubMed]

7. Kwon, B.R.; Wei, B.; Cha, S.Y.; Shang, K.; Zhang, J.F.; Kang, M.; Jang, H.K. Longitudinal Study of the Distribution of Antimicrobial-Resistant Campylobacter Isolates from an Integrated Broiler Chicken Operation. Animals 2021, 11, 246. [CrossRef]

8. Shang, K.; Wei, B.; Jang, H.K.; Kang, M. Phenotypic characteristics and genotypic correlation of antimicrobial resistant (AMR) Salmonella isolates from a poultry slaughterhouse and its downstream retail markets. Food Control. 2019, 100, 35–45. [CrossRef]

9. Pacholewicz, E.; Liakopoulos, A.; Swart, A.; Gortemaker, B.; Dierikx, C.; Havelaar, A.; Schmitt, H. Reduction of extended-spectrum-beta-lactamase- and AmpC-beta-lactamase-producing Escherichia coli through processing in two broiler chicken slaughterhouses. Int. J. Food Microbiol. 2015, 215, 57–63. [CrossRef]

10. Wei, B.; Cha, S.Y.; Kang, M.; Park, I.J.; Moon, O.K.; Park, C.K.; Jang, H.K. Development and application of a multiplex PCR assay for rapid detection of 4 major bacterial pathogens in ducks. Poult. Sci. 2013, 92, 1164–1170. [CrossRef] [PubMed]

11. Wei, B.; Cha, S.Y.; Shang, K.; Zhang, J.F.; Jang, H.K.; Kang, M. Genetic diversity of extended-spectrum cephalosporin resistance in Salmonella enterica and E. coli isolates in a single broiler chicken. Vet. Microbiol. 2021, 254, 109010. [CrossRef]

12. Carattoli, A.; Bertini, A.; Villa, L.; Falbo, V.; Hopkins, K.L.; Threlfall, E.J. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods 2005, 63, 219–228. [CrossRef] [PubMed]

13. Dhillon, R.H.; Clark, J. ESBLs: A Clear and Present Danger? Crit. Care Res. Pract. 2012, 2012, 625170. [CrossRef] [PubMed]

14. Marshall, B.M.; Levy, S.B. Food Animals and Antimicrobials: Impacts on Human Health. Clin. Microbiol. Rev. 2011, 24, 718–733. [CrossRef] [PubMed]

15. Rasschaert, G.; De Zutter, L.; Herman, L.; Heyndrickx, M. Campylobacter contamination of broilers: The role of transport and slaughterhouse. Int. J. Food Microbiol. 2020, 322, 108564. [CrossRef]

16. Projan, M.; von Tippelskirch, P.; Semmler, T.; Guenther, S.; Alter, T.; Roesler, U. Contamination of chicken meat with extended-spectrum beta-lactamase producing Klebsiella pneumoniae and Escherichia coli during scalding and defeathering of broiler carcasses. Food Microbiol. 2019, 77, 185–191. [CrossRef]

17. International Organization for Standardization (ISO). ISO 4832:2006 Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Coliforms—Colony-Count Technique; Technical Committee: Geneva, Switzerland, 2006.

18. Hakeem, M.J.; Lu, X. Survival and Control of Campylobacter in Poultry Production Environment. Front. Cell. Infect. Microbiol. 2020, 10, 615049. [CrossRef]

19. Barilli, V.; Vismarra, A.; Villa, Z.; Bonilauri, P.; Bacci, C. ESBetaL E. coli isolated in pig’s chain: Genetic analysis associated to the phenotype and biofilm synthesis evaluation. Int. J. Food Microbiol. 2019, 289, 162–167. [CrossRef]

20. Gu, H.; Kolewe, K.W.; Ren, D. Conjugation in Escherichia coli Biofilms on Poly(dimethylsiloxane) Surfaces with Microtopographic Patterns. Langmuir 2017, 33, 3142–3150. [CrossRef]

21. Maheshwari, M.; Ahmad, I.; Althubiani, A.S. Multidrug resistance and transferability of blaCTX-M among extended-spectrum beta-lactamase-producing enteric bacteria in biofilm. J. Glob. Antimicrob. Resist. 2016, 6, 142–149. [CrossRef]

22. Botelho, J.; Schultenborg, H. The Role of Integrative and Conjugative Elements in Antibiotic Resistance Evolution. Trends Microbiol. 2021, 29, 8–18. [CrossRef]

23. Lin, Y.T.; Pan, Y.J.; Lin, T.L.; Fung, C.P.; Wang, J.T. Transfer of CMY-2 Cephalosporinase from Escherichia coli to Virulent Klebsiella pneumoniae Causing a Recurrent Liver Abscess. Antimicrob. Agents Chemother. 2015, 59, 5000–5002. [CrossRef]

24. Mo, S.S.; Sunde, M.; Ilag, H.K.; Langsrud, S.; Heir, E. Transfer potential of plasmids conferring extended-spectrum-cephalosporin resistance in Escherichia coli from poultry. Appl. Environ. Microbiol. 2017, 83, e00617–e00654. [CrossRef] [PubMed]

25. Carattoli, A.; Villa, L.; Fortini, D.; Garcia-Fernandez, A. Contemporary IncI1 plasmids involved in the transmission and spread of antimicrobial resistance in Enterobacteriaceae. Plasmid 2018. [CrossRef] [PubMed]
26. Accogli, M.; Fortini, D.; Giufre, M.; Graziani, C.; Dolejska, M.; Carattoli, A.; Cerquetti, M. IncI1 plasmids associated with the spread of CMY-2, CTX-M-1 and SHV-12 in Escherichia coli of animal and human origin. Clin. Microbiol. Infect. 2013, 19, E238–E240. [CrossRef] [PubMed]

27. da Silva, K.C.; Cunha, M.P.V.; Cerdeira, L.; de Oliveira, M.G.X.; de Oliveira, M.C.V.; Gomes, C.R.; Linçopan, N.; Knobl, T.; Moreno, A.M. High-virulence CMY-2 and CTX-M-2-producing avian pathogenic Escherichia coli strains isolated from commercial turkeys. Diagn. Microbiol. Infect. Dis. 2017, 87, 64–67. [CrossRef]

28. Ben Sallem, R.; Ben Slama, K.; Rojo-Bezares, B.; Porres-Osante, N.; Jouini, A.; Klibi, N.; Boudabous, A.; Saenz, Y.; Torres, C. Incl1 plasmids carrying blaCTX-M-1 or blacMY-2 genes in Escherichia coli from healthy humans and animals in Tunisia. Microb. Drug Resist. 2014, 20, 495–500. [CrossRef] [PubMed]

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

30. Ewers, C.; de Jong, A.; Prenger-Berninghoff, E.; El Garch, F.; Leidner, U.; Tiwari, S.K.; Semmler, T. Genomic Diversity and Virulence Potential of ESBL- and AmpC-beta-Lactamase-Producing Escherichia coli Strains From Healthy Food Animals Across Europe. Front. Microbiol. 2021, 12, 519. [CrossRef]

31. Zurfluh, K.; Wang, J.; Klumpp, J.; Nuesch-Inderbinen, M.; Fanning, S.; Stephan, R. Vertical transmission of highly similar blaCTX-M-1-harboring IncI1 plasmids in Escherichia coli with different MLST types in the poultry production pyramid. Front. Microbiol. 2014, 5, 519. [CrossRef]

32. Tamang, M.D.; Nam, H.M.; Kim, T.S.; Jang, G.C.; Jung, S.C.; Lim, S.K. Emergence of extended-spectrum beta-lactamase (CTX-M-15 and CTX-M-14) producing nontyphoid Salmonella with reduced susceptibility to ciprofloxacin among food animals and humans in Korea. J. Clin. Microbiol. 2011, 49, 2671–2675. [CrossRef] [PubMed]