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# Profiling of antimicrobial resistance and plasmid replicon types in β-lactamase producing *Escherichia coli* isolated from Korean beef cattle

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In this study, 78 isolates of *Escherichia coli* isolated from Korean beef cattle farms were investigated for the production of extended-spectrum β-lactamase (ESBL) and/or AmpC β-lactamase. In the disc diffusion test with ampicillin, amoxicillin, cephalothin, ceftiofur, cefotaxime, ceftazidime, and cefoxitin, 38.5% of the isolates showed resistance to all of ampicillin, amoxicillin, and cephalothin. The double disc synergy method revealed that none of the isolates produced ESBL or AmpC β-lactamases. DNA sequencing showed that all isolates encoded genes for TEM-1-type β-lactamase. Moreover, 78.2% of the isolates transferred the TEM-1-type β-lactamase gene via conjugation. In plasmid replicon typing of all donors, IncFIB and IncFIA were identified in 71.4% and 41.0% of plasmids, respectively. In transconjugants, IncFIB and IncFIA were the most frequent types detected (61.5% and 41.0%, respectively). Overall, the present study indicates that selection pressures of antimicrobials on β-lactamases in beef cattle may be low relative to other livestock animals in Korea. Moreover, to reduce selection pressure and dissemination of β-lactamase, the long-term surveillance of antimicrobial use in domestic beef cattle should be established.

**Keywords:** β-lactamase, antimicrobial resistance, *Escherichia coli*, plasmid replicon typing

## Introduction

The prevalence of β-lactam-resistant *Enterobacteriaceae* has increased consistently over the past few decades. *Escherichia* (*E.*) *coli* producing plasmid-mediated AmpC β-lactamases and/or extended-spectrum β-lactamases (ESBLs) has been of particular concern because of their implications in human and food animal health [19]. These strains encode β-lactamases that mediate resistance to β-lactam antimicrobials included penicillins and extended-spectrum cephalosporins such as 3rd and 4th generation cephalosporins [4]. Genes encoding β-lactamases are located on mobile genetic elements, mostly plasmids, which can transfer resistance genes horizontally to non-resistant isolates. Thus, these elements are believed to be responsible for the acquisition and dissemination of β-lactam antimicrobial resistance in the bacterial population.

The incidence of resistance to extended-spectrum β-lactam antimicrobials has increased in Korea [3,17]. Most studies that have been performed to date have focused on the characterization of β-lactamases in human clinical isolates [13,14,21,23,30]. However, there is little information available regarding the prevalence and characteristics of plasmid-mediated AmpC β-lactamases and ESBLs among *E. coli* isolates in the Korean veterinary industry [18,27,31,32]. Furthermore, β-lactamases-producing *E. coli* isolated from beef cattle have rarely been reported in Korea.

Enteric bacteria, especially *E. coli*, derived from livestock animals are potentially infectious pathogens and reservoirs for β-lactamase genes; accordingly, investigations of these microorganisms are necessary for public health. In view of the risk of spreading ESBL and AmpC β-lactamase resistance determinants among *E. coli* isolates, it is important to elucidate the mechanism by which resistance is transferred between isolates. Thus, in the present study, we investigated antimicrobial resistance profiles and plasmid replicon types of ampicillin (AMP)-resistant *E. coli* isolates recovered from the feces of beef cattle with the goal of investigating the transfer of β-lactamase genes and antimicrobial resistance to non-resistant *E. coli*.

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## Materials and Methods

### Bacterial strains

A total of 290 *E. coli* strains were isolated from feces collected from clinically healthy beef cattle during 2011–2012 [29]. Briefly, *E. coli* isolates of this study were isolated from 830 fecal samples collected from healthy beef cattle on eight farms from six different provinces in South Korea. The fecal samples were collected from rectum and pats of cattle and plated onto MacConkey agar (Becton, Dickinson and Company, USA) for selection, then incubated at 37°C for 18 h. From each sample, three to five colonies suspected of being *E. coli* were sub-cultured onto blood agar plates. Isolates were confirmed as *E. coli* by a standard biochemical test and by the Vitek2 system (bioMérieux, France).

### Antimicrobial susceptibility test

For selection of β-lactam-resistant *E. coli*, all isolates were screened by plating on MacConkey agar plates containing AMP (16 μg/mL) because the minimum inhibitory concentration (MIC) value of AMP for *E. coli* was above or at the breakpoint (≥ 32 μg/mL) for AMP resistance [5]. Overall, a total of 78 *E. coli* isolates were selected for characterization of β-lactamases in this study. All 78 *E. coli* isolates were tested using antimicrobial-containing discs according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [5]. The following antibiotics were tested: AMP, 10 μg; amoxicillin (AMX), 20 μg/10 μg; cephalothin (CF), 30 μg; ceftiofur (EFT), 30 μg; cefoxitin (FOX), 30 μg; cefotaxime (CTX), 30 μg; and ceftazidime (CAZ), 30 μg (Oxoid, UK). The MICs of the isolates were also determined by the micro-broth dilution method using the same antibiotics. The MIC test was conducted according to the recommendations of the CLSI [5]. The breakpoint of EFT (MIC ≥ 8 μg/mL) was used based on the results of a previous study [8], because the CLSI guidelines do not include a MIC breakpoint of EFT for *E. coli* of bovine origin. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control organisms in the antimicrobial susceptibility tests and ESBL and/or AmpC β-lactamases in the phenotypic screening test.

### Screening and phenotypic identification of ESBLs and AmpC β-lactamases

A double disc diffusion method was performed with CTX (30 μg)/CTX-clavulanate (30 μg/10 μg; Becton, Dickinson and Company) and CAZ (30 μg)/CAZ-clavulanate (30 μg/10 μg; Becton, Dickinson and Company) to detect ESBL production according to CLSI guidelines [5]. Similarly, plasmid-mediated AmpC β-lactamase production was screened by the cefoxitin-cloxacillin double disc synergy method using FOX (30 μg)/FOX-cloxacillin (30 μg/200 μg; Himedia, India), as described in a previous study [33].

**Table 1.** Primers for the detection of β-lactamase genes used in this study

| β-lactamase(s) targeted | Primers | Sequence | Product size (bp) | Annealing temp (°C) | Reference |
|---|---|---|---|---|---|
| TEM | TEM-F | TCG GGG AAA TGT GCG | 1074 | 62 | [27] |
| | TEM-R | TGC TTA ATC AGT GAG GCA CC | | | |
| SHV | SHV-F | GCC GGG TTA TTC TTA TTT GTC GC | 1016 | 62 | [27] |
| | SHV-R | ATG CCG CCG CCA GTC A | | | |
| OXA | OXA-F | TAT CTACAG CAG CGC CAG TG | 199 | 53 | [8] |
| | OXA-R | CGC ATC AAA TGC CAT AAG TG | | | |
| MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11 | MOX-F | GCT GCT CAA GGA GCA CAG GAT | 520 | 64 | [25] |
| | MOX-R | CAC ATT GAC ATA GGT GTG GTG C | | | |
| LAT-1 to LAT-4, CMY-2 to CMY-7, BIL-1 | CIT-F | TGG CCA GAA CTG ACA GGC AAA | 462 | 64 | [25] |
| | CIT-R | TTT CTC CTG AAC GTG GCT GGC | | | |
| DHA-1, DHA-2 | DHA-F | AAC TTT CAC AGG TGT GCT GGG T | 405 | 64 | [25] |
| | DHA-R | CCG TAC GCA TAC TGG CTT TGC | | | |
| ACC | ACC-F | AAC AGC CTC AGC AGC CGG TTA | 346 | 64 | [25] |
| | ACC-R | TTC GCC GCA ATC ATC CCT AGC | | | |
| MIR-1T, ACT-1 | EBC-F | TCG GTA AAG CCG ATG TTG CGG | 302 | 64 | [25] |
| | EBC-R | CTT CCA CTG CGG CTG CCA GTT | | | |
| FOX-1 to FOX-5b | FOX-F | AAC ATG GGG TAT CAG GGA GAT G | 190 | 64 | [25] |
| | FOX-R | CAA AGC GCG TAA CCG GAT TGG | | | |
| CTX-M universal | CTXMU-F | CGA TGT GCA GTA CCA GTA A | 585 | 60 | [1] |
| | CTXMU-R | TTA GTG ACC AGA ATC AGC GG | | | |

**Detection of β-lactamase-encoding genes**

PCR amplification of genes of the ESBL ($bla_{TEM}$, $bla_{SHV}$, $bla_{OXA}$, and $bla_{CTX-M}$) and plasmid-mediated AmpC was carried out as previously described [2,9,25,27]. The primers used to detect β-lactamases in this study are shown in Table 1. The DNA templates used in this study were prepared by the boiling method. In all PCR amplifications, distilled water was used as a negative control. A positive control organism was not used in this assay as all DNA products were sequenced by a dye-termination sequencing system using an automatic sequencer (Macrogen, Korea). Homologous sequence searches were performed against the GenBank database using the BLAST tool of the National Center for Biotechnology Information (NCBI, USA) website.

**Conjugation assay**

To determine the transferability of the β-lactamase-encoding genes, a conjugation assay was conducted. A mixed broth

**Table 2.** Primers for analysis of plasmid replicon types used in this study

| Replicons | Target sites | Primer sequence | | Annealing temp (°C) | Product size (bp) |
|---|---|---|---|---|---|
| | | Direction | Sequence (5′ to 3′) | | |
| T | *repA* | F | TTG GCC TGT TTG TGC CTA AAC CAT | 60 | 750 |
| | | R | CGT TGA TTA CAC TTA GCT TTG GAC | | |
| P | Iterons | F | CTA TGG CCC TGC AAA CGC GCC AGA AA | 60 | 534 |
| | | R | TCA CGC GCC AGG GCG CAG CC | | |
| A/C | *repA* | F | GAG AAC CAA AGA CAA AGA CCT GGA | 60 | 465 |
| | | R | ACG ACA AAC CTG AAT TGC CTC CTT | | |
| FIC | *repA2* | F | GTG AAC TGG CAG ATG AGG AAG G | 60 | 262 |
| | | R | TTC TCC TCG TCG CCA AAC TAG AT | | |
| B/O | RNAI | F | GCG GTC CGG AAA GCC AGA AAA C | 60 | 159 |
| | | R | TCT GCG TTC CGC CAA GTT CGA | | |
| Y | *repA* | F | AAT TCA AAC AAC ACT GTG CAG CCT G | 60 | 765 |
| | | R | GCG AGA ATG GAC GAT TAC AAA ACT TT | | |
| FIB | *repA* | F | GGA GTT CTG ACA CAC GAT TTT CTG | 60 | 702 |
| | | R | CTC CCG TCG CTT CAG GGC ATT | | |
| FIA | Iterons | F | CCA TGC TGG TTC TAG AGA AGG TG | 60 | 462 |
| | | R | GTA TAT CCT TAC TGG CTT CCG CAG | | |
| FIIA | *repA* | F | CTG TCG TAA GCT GAT GGC | 60 | 270 |
| | | R | CTC TGC CAC AAA CTT CAG C | | |
| W | *repA* | F | CCT AAG AAC AAC AAA GCC CCC G | 60 | 242 |
| | | R | GGT GCG CGG CAT AGA ACC GT | | |
| K/B | RNAI | F | GCG GTC CGG AAA GCC AGA AAA C | 60 | 160 |
| | | R | TCT TTC ACG AGC CCG CCA AA | | |
| L/M | RepA,B,C | F | GGA TGA AAA CTA TCA GCA TCT GAA G | 60 | 785 |
| | | R | CTG CAG GGG CGA TTC TTT AGG | | |
| HI2 | Iterons | F | TTT CTC CTG AGT CAC CTG TTA ACA C | 60 | 644 |
| | | R | GGC TCA CTA CCG TTG TCA TCC T | | |
| N | *repA* | F | GTC TAA CGA GCT TAC CGA AG | 60 | 559 |
| | | R | GTT TCA ACT CTG CCA AGT TC | | |
| HI1 | *parA-parB* | F | GGA GCG ATG GAT TAC TTC AGT AC | 60 | 471 |
| | | R | TGC CGT TTC ACC TCG TGA GTA | | |
| X | oriɣ | F | AAC CTT AGA GGC TAT TTA AGT TGC TGA T | 60 | 376 |
| | | R | TGA GAG TCA ATT TTT ATC TCA TGT TTT AGC | | |
| Frep | RNAI/*repA* | F | TGA TCG TTT AAG GAA TTT TG | 60 | 270 |
| | | R | GAA GAT CAG TCA CAC CAT CC | | |
| I1 | RNAI | F | CGA AAG CCG GAC GGC AGA A | 60 | 139 |
| | | R | TCG TCG TTC CGC CAA GTT CGT | | |

F, forward; R, reserve.

culture mating method in a previous study was applied with sodium azide-resistant *E. coli* J53AzR as a recipient strain, with some modifications [27]. Single colonies of donor and recipient isolates were incubated in tryptic soy broth (TSB; Becton, Dickinson and Company) and grown at $37^{o}$C for 20 h. The donor and recipient strains were grown in TSB for 8 hrs, after which the cultures were mixed at a ratio of 1: 2 and incubated at $37^{o}$C for 20 h. Transconjugants were selected on Mueller-Hinton agar (Becton, Dickinson and Company) supplemented with AMP (100 μg/mL) and sodium azide (200 μg/mL). The conjugation frequency of each isolate was calculated as the number of CFU transconjugants per CFU donor. In addition, transfer of the genes was confirmed by PCR amplification of specific genes in the transconjugants.

### Typing of plasmid replicons

For typing plasmid replicons, PCR was performed using DNA extracted from all donor and transconjugant strains. The primers used in this study targeted 18 different replicons (Table 2), as described previously [16].

## Results

### Antimicrobial resistance

Resistance to AMP and AMX was observed in all isolates, and 30 isolates (38.5%) were resistant to CF. None of the isolates showed resistance to any of the extended-spectrum β-lactams used in the test (EFT, CAZ, CTX, and FOX) (Table 3). The MIC values of the different β-lactams tested for the 78 *E. coli* isolates are shown in Table 3. All isolates were highly resistant to AMP (MIC > 1024 μg/mL) and AMX (MIC > 1024 μg/ml). Cephalothin resistance (MIC ≥ 32 μg/mL) was detected in 32 isolates (41.0%). None of the isolates was resistant to EFT (MIC ≤ 4 μg/mL), CAZ (MIC ≤ 8 μg/mL), CTX (MIC ≤ 2 μg/mL), or FOX (MIC ≤ 8 μg/mL) (Table 3). However, intermediate resistance to EFT (MIC = 4 μg/mL), CAZ (MIC = 8 μg/mL), and CTX (MIC = 2 μg/mL) was detected in 39.7%, 17.9%, and 46.2% of the isolates, respectively. The resistance patterns of the isolates were [AMP-AMX] (61.5%) and [AMP-AMX-CF] (38.5%).

### Screening of ESBL and AmpC β-lactamase production

None of the isolates were positive for ESBL or AmpC β-lactamase production. In the MIC test, none of the isolates were resistant to CTX, CAZ, or FOX, even though 36 (46.2%), 14 (17.9%), and 4 (5.1%) of the *E. coli* isolates showed intermediate MIC values against CTX (MIC, 2 μg/mL), CAZ (MIC, 8 μg/mL), and FOX (MIC, 8 μg/mL), respectively (Table 3).

### Molecular characterization of β-lactamase-encoding genes

All 78 *E. coli* isolates harbored a TEM-type gene. None of the genes encoding the ESBLs ($bla_{SHV}$, $bla_{OXA}$, and $bla_{CTX\text{-}M}$) or pAmpC β-lactamases were found in any of the isolates. Sequence analysis identified TEM-1-type β-lacatamase in all isolates.

### Transferability of β-lactamase resistance and plasmid replicon analysis

Plasmid replicon typing and conjugal transferability of plasmids revealed that the $bla_{TEM\text{-}1}$, gene for β-lactamase resistance was transferred in 59 (75.6%) of the isolates (Table 4). The transfer frequency of the isolates ranged from $1.29 \times 10^{-6}$ to $9.22 \times 10^{-4}$. Plasmid replicon typing of the transconjugants was performed to identify the transfer of plasmids in *E. coli* carrying the TEM-1 gene. The prevalence of the plasmid replicon type of the donor isolates was as follows: IncFIB (71.8%); IncFIA (41.0%); IncP (34.6%); Frep (29.5%); IncY (29.5%); IncI1 (28.2%); IncN (15.4%); IncB/O (10.3%) and IncHI1 (1.3%). Among the 10 plasmids detected from the

**Table 3.** Antimicrobial susceptibility of 78 *Escherichia coli* isolates to β-lactam antimicrobial agents

| Antimicrobials | Phenotype of disc diffusion method | | | MIC (μg/mL) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R (%) | I (%) | S (%) | < 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 | > 1024 |
| Ampicillin | 100 | 0 | 0 | | | | | | | | | | | | | 78 |
| Amoxicillin | 100 | 0 | 0 | | | | | | | | | | | | | 78 |
| Cephalothin | 38.5 | 61.5 | 0 | | | | | 36 | 10 | 18 | 12 | 2 | | | | |
| Ceftiofur | 0 | 0 | 100 | 4 | 32 | 11 | 31 | | | | | | | | | |
| Ceftazidime | 0 | 0 | 100 | 3 | 3 | 4 | 54 | 14 | | | | | | | | |
| Cefotaxime | 0 | 0 | 100 | 2 | 40 | 36 | | | | | | | | | | |
| Cefoxitin | 0 | 0 | 100 | | 3 | 59 | 12 | 4 | | | | | | | | |

MIC, minimum inhibitory concentration; R, resistant; I, intermediate; S, susceptible.

**Table 4.** Profile of plasmid replicon typing and transferability of 78 *Escherichia coli* isolates

| Number of replicons | Donor replicon | Number of strains | Transferability | Transfer frequency | Replicon of transconjugant | Transfer of β-lactamase |
|---|---|---|---|---|---|---|
| 1 | B/O | 1 | + | $2.52 \times 10^{-4}$ | B/O | |
| | FIB | 1 | + | $4.01 \times 10^{-5}$ | FIB | TEM-1 |
| | I1 | 2 | + (2/2) | $3.68 \times 10^{-5}$–$6.81 \times 10^{-5}$ | I1 | TEM-1 |
| | N | 2 | – | | | |
| | Frep | 2 | + (1/2) | $3.14 \times 10^{-4}$ | Frep | TEM-1 |
| | FIA | 5 | + (4/5) | $9.09 \times 10^{-6}$–$1.01 \times 10^{-4}$ | FIA | TEM-1 |
| | P | 5 | – | | | |
| 2 | P-FIA | 1 | + | $3.91 \times 10^{-5}$ | FIA | TEM-1 |
| | P-I1 | 1 | – | | | |
| | FIB-I1 | 1 | + | $9.81 \times 10^{-6}$ | FIB-I1 | TEM-1 |
| | FIB-Y | 1 | + | $3.27 \times 10^{-5}$ | FIB | TEM-1 |
| | FIB-Frep | 1 | + | $7.71 \times 10^{-6}$ | FIB | TEM-1 |
| | FIA-FIB | 2 | + (2/2) | $2.91 \times 10^{-5}$–$7.11 \times 10^{-5}$ | FIA-FIB | TEM-1 |
| | FIB-N | 3 | – | | | |
| 3 | B/O-P-FIB | 1 | + | $2.52 \times 10^{-5}$ | B/O-FIB | TEM-1 |
| | P-FIA-FIB | 1 | + | $4.45 \times 10^{-5}$ | FIA-FIB | TEM-1 |
| | P-FIB-Y | 1 | + | $3.62 \times 10^{-5}$ | FIB | TEM-1 |
| | P-FIB-Frep | 1 | + | $9.22 \times 10^{-4}$ | FIB | TEM-1 |
| | FIB-Y-Frep | 1 | + | $8.32 \times 10^{-5}$ | FIB | TEM-1 |
| | FIB-I1-Frep | 2 | + (2/2) | $6.24 \times 10^{-6}$–$3.33 \times 10^{-5}$ | FIB-I1, FIB-Frep | TEM-1 |
| | FIB-Y-I1 | 5 | +(5/5) | $1.29 \times 10^{-6}$–$5.24 \times 10^{-4}$ | FIB, I1, FIB-I1, FIA-FIB-I1 | TEM-1 |
| | FIA-FIB-Frep | 5 | + (5/5) | $8.24 \times 10^{-6}$–$4.48 \times 10^{-5}$ | FIA, FIB, FIA-FIB-Frep, | TEM-1 |
| | P-FIB-I1 | 6 | + (5/6) | $9.24 \times 10^{-6}$–$3.31 \times 10^{-5}$ | FIB-I1 | TEM-1 |
| | FIA-FIB-Y | 8 | + (8/8) | $9.57 \times 10^{-6}$–$1.44 \times 10^{-5}$ | FIA-FIB | TEM-1 |
| 4 | B/O-FIB-Frep-N | 1 | + | $2.56 \times 10^{-5}$ | B/O-Frep | TEM-1 |
| | FIA-FIB-Y-HI1 | 1 | + | $2.78 \times 10^{-5}$ | FIA-FIB | TEM-1 |
| | P-FIB-I1-Frep | 2 | + (2/2) | $3.01 \times 10^{-5}$–$1.19 \times 10^{-4}$ | FIB, FIB-I1-Frep | TEM-1 |
| | B/O-P-FIB-Frep | 2 | + (1/2) | $5.78 \times 10^{-4}$ | B/O | |
| | B/O-P-I1-Frep | 2 | + (2/2) | $4.27 \times 10^{-5}$–$7.79 \times 10^{-5}$ | I1-Frep | TEM-1 |
| | P-FIA-FIB-Frep | 3 | + (3/3) | $3.33 \times 10^{-5}$–$1.91 \times 10^{-4}$ | FIA-FIB-Frep | TEM-1 |
| | FIA-FIB-Y-N | 6 | + (6/6) | $2.52 \times 10^{-6}$–$4.49 \times 10^{-5}$ | FIA-FIB | TEM-1 |
| 5 | B/O-P-FIB-I1-Frep | 1 | + | $5.62 \times 10^{-4}$ | FIB-Frep | TEM-1 |
| 0 | None | 1 | – | | | |

isolates, the main plasmid for the horizontal dissemination of $bla_{TEM-1}$ in *E. coli* isolated from beef cattle was the IncFIB (Table 4). Plasmid replicon typing revealed that all donor isolates exhibited 32 different replicon combinations. The most frequent combination was [FIA-FIB-Y], which was detected in eight isolates (Table 4). For transconjugants, a total of five classes of replicon were detected. IncFIB and IncFIA were the most frequently detected replicons, being found either alone or in combination at ratios of 61.5% and 41.0%, respectively. The prevalence of the remaining plasmid replicons of transconjugants was as follow: IncI1 (17.9%); Frep (16.7%) and IncB/O (5.1%). PCR revealed that all 59 transconjugants harbored TEM-1-type β-lacatamase transferred from the donors.

## Discussion

In the present study, we conducted phenotypic and genotypic characterization of β-lactamase of *E. coli* strains isolated from Korean beef cattle farms from 2011 to 2012. None of the *E. coli* isolates were found to produce ESBL and/or AmpC β-lactamase.

High MIC values for AMP in *E. coli* isolated from calves with diarrhea and dairy cattle were reported in previous studies [20,28]. In the present study, the extremely high resistance to AMP (MIC $>$ 1024 μg/mL resistance, 100%) and AMX (MIC $>$ 1024 μg/mL resistance, 100%) of these *E. coli* isolates might have been caused by selection pressures from their excessive use in beef cattle farms over the last decade [1]. Additionally, the use of β-lactam antimicrobials, such as penicillins and

cephems, has increased gradually [1]. In addition, the antimicrobial resistance to CF of the *E. coli* isolates used in this study was high, with 32 (41.0%) isolates showing resistance to CF (MIC $\geq$ 32 μg/mL), and this resistance was much higher than that of *E. coli* (1.0%) in a previous national report [1]. A considerable number of isolates exhibited intermediate resistance to CTX (n = 36), EFT (n = 31), and CAZ (n = 14), although none of the isolates in this study were identified as resistant to these compounds (Table 3). *E. coli* isolates showing intermediate resistance to these compounds may acquire resistance to β-lactams by selection pressure if they are exposed to continuous use of antimicrobials.

In this study, no ESBL- and/or AmpC β-lactamase-producing *E. coli* isolates were detected, which is consistent with the results of a previous study showing a low prevalence ( < 2%) of β-lactamase-producing *E. coli* isolates [18,31,32]. Although recent reports indicated that there are various types of ESBL- and AmpC β-lactamase-producing *Enterobacteriaceae* [11,12,14,21,24], only TEM-1-type β-lactamase was detected in the present study. These findings suggest that less third- and fourth-generation cephalosporins might be used in the production of Korean beef cattle than in the human population and production of other livestock. In the present study, PCR and sequencing results revealed that all AMP-resistant isolates were only associated with TEM-1-type β-lactamase, which is known to be widely distributed in Korea [22,27]. These results are in agreement with those of a previous study, which showed that most of the AMP-resistant *E. coli* harbored the TEM-1 β-lactamase gene as the only plasmid-mediated β-lactamase [6].

Continuous selective pressure exerted by β-lactams is an important reason for occurrence of ESBL- and AmpC β-lactamase determinants [10]. Similarly, genetically non-resistant strains might be able to acquire resistance plasmids, either randomly or specifically, due to constant antimicrobial use, leading to widespread occurrence of resistance plasmids [26]. Replicon typing of the transconjugant of *E. coli* isolates revealed that the IncFIA and IncFIB plasmids, which are commonly found in the fecal flora of humans and animals, were most frequently detected [7]. We found that strains that carried F plasmid (IncFIB, IncFIA and Frep) and I1 either alone or combination had transferred the TEM-1-type β-lactamase. These results suggest that blaTEM-1 gene, a primitive type of β-lactamase encoding gene, is harbored by these kind of plasmids and associated with old type β-lactams such as AMP and AMX [15] Two isolates that carried IncB/O did not transfer TEM-1-type β-lactamase to the recipients.

When compared to other veterinary studies, our results are unusual as no resistance to cephems was found and only one kind of β-lactamase was detected. These results suggest that the present selection pressure of antimicrobial use on β-lactamases in beef cattle may be relatively low in comparison to other livestock in Korea. However, increased exposure to antimicrobials could increase selection pressure for β-lactamases, which presents a critical risk to human and animal health. Thus, the use of β-lactam antimicrobials such as extended-spectrum cephalosporin should be restricted. In addition, monitoring the use of antimicrobials and assessment of antimicrobial resistance mechanisms in the bacteria of beef cattle could reduce selection pressure and may help enhance treatment for both humans and animals.

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## Conflict of Interest

There is no conflict of interest.

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