Molecular characterization of fluoroquinolone-resistant *Escherichia coli* from broiler breeder farms

Kwang Won Seo,*† and Young Ju Lee*†

*College of Veterinary Medicine & Zoonoses Research Institute, Kyungpook National University, Daegu 41566, Republic of Korea; and †Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, MS 39762, USA

**ABSTRACT** Fluoroquinolones (FQs) have been used effectively antimicrobial agents of choice for treatment of various infections caused by *E. coli* and FQs-resistance of *E. coli* from broiler breeders has been implicated in its vertical transmission to their offspring. The objective of this study investigated the phenotypic and genotypic characteristics of FQ-resistant *E. coli* isolates from broiler breeder farms in Korea. A total of 106 FQ-resistant *E. coli* isolates were tested in this study and all isolates had mutations in quinolone resistance determining regions; all (100%) had mutations in *parE* and *parC*, and none had mutations in *gyrB*. The predominant mutation type was double mutation in *gyrA* (S83L and D87N), and all FQ-resistant *E. coli* isolates that had mutations in *parC* or *parE* also had double mutations in *gyrA*. Especially, FQ-resistant *E. coli* isolates which possessed double mutations in *gyrA* in combination with double mutations in *parC* or single mutations in both *parC* and *parE* were shown high levels of minimum inhibitory concentrations range. Of the 23 plasmid-mediated quinolone resistance (PMQR)-positive *E. coli* isolates, *qnrS* was detected in 10 (9.4%) isolates, and followed by *qnrA* (7 isolates, 6.6%), *qnrB* (4 isolates, 3.8%), and *aac(6’)-Ib-cr* (2 isolates, 1.9%). Sixteen (69.6%) of the 23 PMQR-positive *E. coli* isolates harbored class 1 integrons with four different gene cassette arrangements and total of 9 plasmid replicon types were also identified in 23 PMQR-positive *E. coli* isolates. This is the first study to investigate the prevalence and characteristics of FQ-resistant and PMQR-positive *E. coli* isolated from the broiler breeder in Korea; it supports that constant monitoring and studies at the broiler breeder level are required to prevent the pyramidal transmission of FQ-resistant *E. coli*.

**Key words:** *Escherichia coli*, fluoroquinolone, antimicrobial resistance, broiler parent stock, PMQR

© 2021 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Received March 6, 2021.

Accepted May 3, 2021.

*Corresponding author: youngju@knu.ac.kr

**INTRODUCTION**

Fluoroquinolones (FQs) have been used effectively antimicrobial agents of choice for treatment of various infections caused by *E. coli* or other gram-negative bacteria. Because of clinical importance in both human and animal medicine, the World Health Organization has classified FQs as “critically important antimicrobials (WHO, 2017). However, the continuous use of FQs in livestock can lead to the emergence and maintenance of FQ-resistant bacteria, and it is considered a significant public health threat (Wasyl et al., 2013, Xu et al., 2015). Especially, since enrofloxacin have been introduced to the poultry industry in Korea in 1987, FQ-resistant *E. coli* have developed over the time (Hu et al. 2017; Seo and Lee, 2020).

FQ-resistance is mainly due to chromosomal mutations that alter the drug target enzymes DNA gyrase (*gyrA* and *gyrB*) and DNA topoisomerase IV (*parC* and *parE*) (Jacoby, 2005). Moreover, 3 different plasmid-mediated quinolone resistance (PMQR) determinants have been described: the *qnr* genes that protect the DNA gyrase and topoisoamerase IV from quinolone inhibition, the *aac(6’)-Ib-cr* gene that an aminoglycoside acetyltransferase that confers reduced susceptibility to ciprofloxacin, and the *qepA* gene that the major facilitator superfamily-type quinolone efflux pump decreasing susceptibility to quinolones (Liu et al., 2012). Although PMQR genes confer low-level resistance to FQ, they can facilitate the selection of mutations in gyrase and topoisoamerase genes which results in high-level FQ-resistance (Yang et al., 2008).

https://doi.org/10.1016/j.psj.2021.101250

2021 Poultry Science 100:101250
The broiler industry has a pyramidal structure in which grandparent stock on the top through breeding chickens parent stock that produce eggs for the produce the broiler chickens on the bottom. In this structure, antimicrobial resistant bacteria and drug-resistance genes can be vertically transmitted through the broiler breeding chain. Although studies from several countries have documented the prevalence and characteristics of FQ-resistance in commercial broiler level (Taylor et al., 2008; Abdi-Hachesoo et al., 2017; Nishikawa et al., 2019), there is still limited information regarding the molecular characteristics of FQ-resistant and PMQR-positive isolates at the broiler breeding level. Therefore, this study investigated the phenotypic and genotypic characteristics of FQ-resistant E. coli isolates from broiler breeder farms in Korea.

**MATERIALS AND METHODS**

**Sampling**

Feces and dust were sampled from nine broiler breeding farms including 69 flocks (20 wk of age) between 2016 and 2018 in accordance with the standards set by the National Poultry Improvement Plan (United States Department of Agriculture USDA, 2011). Briefly, 15 different spots were swabbed per flock in order to collect 10 g of dust sample using surgical gauze moistened with 12 mL of sterile double strength skim milk (Fluka, Neu-Ulm, Germany). Approximately 10 g of feces were also sampled from 15 different locations. Samples were transported to the laboratory in a cooler and stored at 4°C until use.

**Bacterial Identification**

The samples were individually inoculated into 225 mL of mEC (Merck, Darmstadt, Germany) and incubated at 37°C for 20 to 24 h. Pre-enriched mEC was streaked onto MacConkey agar (BD Bioscienes, Sparks, MD) plates and incubated at 37°C for 24 h. Five typical colonies selected from each sample were identified by PCR as previously described (Candrian et al., 1991), and plated on Mueller-Hinton agar (BD Biosciences, Sparks, MD) plates supplemented with 4 μg/mL ciprofloxacin (Sigma-Aldrich, St. Louis, MO) to select FQ-resistant E. coli. If isolates of the same origin showed the same antimicrobial susceptibility patterns, only one isolate was randomly chosen and included in the analysis. As a result, a total of 106 FQ-resistant E. coli were tested in this study (Table 1).

### Antimicrobial Susceptibility Testing

All FQ-resistant E. coli isolates were investigated for their antimicrobial resistance with the disc diffusion test using the following discs (BD Biosciences): amoxicillin-clavulanate (20/10 μg), ampicillin (10 μg), cefazolin (30 μg), cephalothin (30 μg), cefadroxil (30 μg), cefoxitin (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), imipenem (10 μg), nalidixic acid (30 μg), tetracycline (30 μg), and trimethoprim-sulfamethoxazole (1.25/23.75 μg). Minimum inhibitory concentrations (MICs) ranging from 0.06 to 512 mg/L to nalidixic acid, ciprofloxacin, and enrofloxacin (Sigma-Aldrich) were determined using standard agar dilution methods according to recommendations of the Clinical & Laboratory Standards Institute (CLSI, 2015, 2020). E. coli ATCC 25922 was included as a quality control. Multi-drug-resistance (MDR) was defined as acquired resistance to at least one agent in 3 or more antimicrobial classes (Magiorakos et al., 2012).

### Identification of Mutations in QRDRs and Detection of PMQRs

PCR was carried out to amplify the target genes (gyrA, gyrB, parC, and parE) in quinolone resistance determining regions (QRDRs) to identify mutations in 106 FQ-resistant E. coli isolates using primers and conditions described previously (Fendukly et al., 2003; Dutta et al., 2005; Bai et al., 2012). The PCR products were purified using GFX PCR DNA and the Gel band purification kit (Amersham Bioscience, Freiburg, Germany), and sequenced by automatic sequencer (Cosmogenetech, Seoul, Korea). The sequences were confirmed with those in the GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST) program available through the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST). PMQR genes (qnrA, qnrB, qnrC, qnrD, qnrS, aac(6’)-Ib-cr, and qepA) were also detected by PCR amplification and sequencing analysis, as described in previous studies (Yu et al., 2015).

### Table 1. Distribution of 106 ciprofloxacin-resistant E. coli isolated from 9 broiler breeder farms.

| Broiler breeder farms | I | II | III | IV | V | VI | VII | VIII | IX | Total |
|-----------------------|---|----|-----|----|---|----|-----|------|----|-------|
| No. of flocks tested  | 6 | 9  | 10  | 17 | 7 | 7  | 5   | 5    | 3  | 69    |
| No. of positive flocks (%) | 5 (83.3) | 8 (88.9) | 9 (90.0) | 13 (76.5) | 7 (100.0) | 5 (71.4) | 4 (80.0) | 3 (100.0) | 4 (80.0) | 58 (84.1) |
| No. of positive flocks (%) | 9 | 12 | 18  | 22 | 14| 10 | 8   | 6    | 7  | 106   |
| No. PMQR-positive E. coli | 2 | 4  | 5   | 5  | 2 | 2  | 0   | 0    | 3  | 23    |

1If several isolates from same origin showed the same antimicrobial susceptibility patterns, only one isolate was included.
2PMQR, plasmid-mediated quinolone resistance.
Plasmid Replicon Typing and Detection of Integrons and Gene Cassettes

For plasmid replicon typing and detection of integrons and gene cassettes, PCR was performed using DNA extracted from 23 PMQR-positive \textit{E. coli} isolates. The primers used in this study targeted 18 different replicons (Johnson et al., 2007) and class 1 and 2 integrons (Ng et al., 1999; Sáenz et al., 2004). Gene cassettes were tested for integron-positive isolates (Ng et al., 1999; Sáenz et al., 2004). The PCR products of the gene cassettes were sequenced as described above.

Transfer of Resistance Genes by Conjugation

To determine the transferability of PMQR genes, conjugation assays were performed using the broth mating method, with \textit{E. coli} J53 used as the recipient as previously described (Tamang et al., 2012). Transconjugants were selected on MacConkey agar (BD Biosciences) plates containing sodium azide (100 \(\mu\)g/mL; Sigma-Aldrich) and ampicillin or tetracycline (100 \(\mu\)g/mL; Sigma-Aldrich). All transconjugants were tested for the presence of PMQR genes, as described above.

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed on PMQR-positive \textit{E. coli} isolates by digesting the genomic DNA using the \textit{XbaI} restriction enzyme (Takara Bio Inc., Shiga, Japan) according to the standard protocol of the Center for Disease Control and Prevention and CHEF-MAPPER apparatus (Bio-Rad Laboratories, Hercules, CA), as previously described (Liu et al., 2007). Gel images were analyzed using InfoQuest FP software ver. 4.5 (Bio-Rad). The similarity matrix was expressed graphically by an unweighted average linkage.

RESULTS

Antimicrobial Resistance Profile

The antimicrobial resistance patterns of FQ-resistant \textit{E. coli} isolated from broiler breeder farms is shown in Figure 1. FQ-resistant \textit{E. coli} isolates showed the highest resistance to quinolones (100.0%) and cephalosporins (100.0%) followed by penicillins (90.6%), tetracyclines (90.6%), folate pathway inhibitors (77.4%), phenicols (72.6%), \(\beta\)-lactam/\(\beta\)-lactamase inhibitor combinations (25.8%), aminoglycosides (13.2%), and carbapenems (5.7%). Also, all FQ-resistant \textit{E. coli} isolates were identified as having MDR against 3 to 10 classes of antimicrobial agents. The rate of resistance to 8 antimicrobial classes was the highest at 34.0% and 1 (0.9%) FQ-resistant \textit{E. coli} isolate showed resistance to 10 classes.

Presence of Amino Acid Substitutions in QRDRs in FQ-Resistant \textit{E. coli}

All 106 FQ-resistant \textit{E. coli} isolates showed the mutation in \textit{gyrA}. But, 89 (84.0%) isolates showed the mutation with \textit{parE}, and 8 (7.5%) isolates showed the mutations with \textit{parC} and \textit{parE}, simultaneously (Table 2). The high \textit{gyrA} amino acid substitutions were S83L (99 isolates, 93.4%) and D87N (75 isolates, 70.8%), and 75 isolates showed double mutations of S83L and D87N. The highest \textit{parC} substitution were S80I (74 isolates, 69.8%), but 25 isolates also showed double mutations of S80I and E84A. In \textit{parE} mutations, I464F (5 isolates) and S458A (3 isolates) were observed. The \textit{gyrB} mutations were not detected in any of the isolates in this study. MICs range of isolates with double mutations in \textit{gyrA} were relatively higher than those of other isolates with single mutations in \textit{gyrA}. Especially, FQ-resistant \textit{E. coli} isolates with a high level of MICs rage (\(\geq 64\) mg/L for ciprofloxacin and \(\geq 128\) mg/L for enrofloxacin) were shown to carry double mutations in \textit{gyrA} in combination with other antimicrobial resistance genes.

Figure 1. Antimicrobial resistance classes (A) and spectrum (B) of 106 fluoroquinolone-resistant \textit{E. coli} isolated from broiler breeder farms. Abbreviations: AMGs, aminoglycosides; BL/BLICs, \(\beta\)-lactam/\(\beta\)-lactamase inhibitor combinations; CARs, carbapenems; CEPs, cephalosporins; FPIs, folate pathway inhibitors; PCNs, penicillins; PHs, phenicols; Qs, quinolones; TETs, tetracyclines.
with mutations in parC. PMQR genes were detected in 23 (21.7%) of the 106 FQ-resistant E. coli isolates. The qnrS was detected in 10 isolates (9.4%), followed by qnrA (7 isolates, 6.6%), qnrB (4 isolates, 3.8%), and aac(6\(^{-}\))Ib-cr (2 isolates, 1.9%).

### Characteristics of PMQR-Positive E. coli

The phenotypic and genotypic characteristics of the 23 PMQR-positive isolates among the 106 FQ-resistant E. coli isolates are shown in Figure 2. Sixteen (69.6%) isolates were found to have class 1 integrons, with the following 4 types of gene cassettes, dfrA1 (6 isolates), dfrA17 (3 isolate), aadA2 (2 isolate), and dfrA1+aadA1 (1 isolate). Four isolates did not carry any of the gene cassettes. A total of 9 plasmid replicon types were also identified in 23 PMQR-positive E. coli isolates. The most common plasmid replicon was FIB (12 isolates, 52.2%), followed by FIA (9 isolates, 39.1%). Transferability of PMQR genes was only identified in ten (43.5%) isolates among 23 PMQR-positive E. coli isolates.

#### PFGE Analysis

In determination of the epidemiological genetic relationships by PFGE (Figure 2), 18 PFGE patterns showing 85% similarity were observed in 23 PMQR-positive E. coli isolates. In particular, isolates that included 3 PFGE patterns (PEP003, PEP011, and PEP018) were

![Figure 2](image-url)

**Figure 2.** Pulsed-field gel electrophoresis patterns of XbaI-digested total DNA of 23 PMQR-positive E. coli isolated from broiler breeder farms. The black color indicates that the trait is present, and the gray color indicates that the trait is absent. Self-transfer of carrying PMQR genes in conjugation experiments. Abbreviations: AM, ampicillin; AMC, amoxicillin-clavulanate; AMR, antimicrobial resistance; C, chloramphenicol; CIP, ciprofloxacin; CZ, cefazolin; CF, cephalothin; CFR, cefadroxil; FOX, cefoxitin; G, gentamicin; IPM, imipenem; NA, nalidixic acid; PMQR, plasmid-mediated quinolone resistance; SXT, sulfamethoxazole/trimethoprim; QRDR, quinolone-resistance determining region; TE, tetracycline.

### Table 2. Amino acid changes in the QRDRs, MICs and PMQR determinants of 106 fluoroquinolone-resistant E. coli isolates.

| QRDR mutations | MICs range (µg/mL) | PMQR genes (No. of isolates) | No. of fluoroquinolone-resistant E. coli (%) |
|----------------|--------------------|-----------------------------|------------------------------------------|
| gyrA gyrB parC parE | NA CIP ENR | qnrA (4), qnrB (2) | 4 (3.8) |
| S83L/D87N WT S80I/E84A I464F | >512 256 256 | - | 21 (19.8) |
| S83L/D87N WT S80I/E84G WT | >512 64-128 128-256 | qnrA (7), 6.6% | 2 (1.9) |
| S83L/D87N WT S80I | >512 128 | qnrA (1) | 1 (0.9) |
| S83L/D87N WT S80I | >512 128-256 | qnrS (3) | 9 (8.5) |
| S83L/D87N WT S80I | >512 32-64 | qnrS (2) | 10 (9.4) |
| S83L/D87N WT S80I | >512 32 | qnrA (1) | 7 (6.6) |
| S83L/D87E WT WT | >512 8 | - | 5 (4.7) |
| S83L WT WT | >512 4 | - | 2 (1.9) |
| S83L WT WT | >512 4 | - | 7 (6.6) |
| 8 | <0.06 | <0.06 | ATCC 25922 |

1QRDR, quinolone-resistance determining region; WT, wild type.
2MICs, minimum inhibitory concentrations; NA, nalidixic acid; CIP, ciprofloxacin; ENR, enrofloxacin.
3PMQR, plasmid-mediated quinolone resistance; -, not detected.
originated from the same broiler breeder farm with the same antimicrobial resistance genes, QRDR mutation, and plasmid replicon types, and showed similar antimicrobial resistance patterns.

**DISCUSSION**

FQs are highly effective antimicrobial class with many advantages including high oral absorption, large volume of distribution, and broad-spectrum antimicrobial activity (Patel and Goldman, 2016). In Korea, the mass medication of poultry with FQs is still permitted, and the sale volume of enrofloxacin is the highest among all antimicrobials (APQA, 2017). However, resistance to FQs has emerged following their widespread use in poultry farms; thus, FQ-resistant *E. coli* isolates can be spread in poultry production pyramid (Seo and Lee, 2020). In this study, 106 FQ-resistant *E. coli* isolates showed co-resistance to cephems (100.0%) penicillins (90.6%), and tetracyclines (90.6%). Especially, all isolates showed MDR against more than 3 antimicrobial agents, and nine isolates showed resistance to more than 9 classes. These results are consistent with those of recent studies showing co-association of resistance to other classes of antimicrobials and high MDR rates among FQ-resistant *E. coli* (Mitra et al., 2019, Seo and Lee, 2020). It is because FQ-resistant *E. coli* has plasmids harboring resistant genes to diverse classes of antimicrobials including PMQR genes (Mitra et al., 2019).

In this study, all FQ-resistant *E. coli* isolate showed amino acid exchanges at *gyrA*. Especially, isolates that had *parC* and *parE* mutations also had double mutations in *gyrA*. Heisig et al. reported that because the DNA gyrase activity is more sensitive to quinolones than that of DNA topoisomerase IV, *gyrA* becomes the primary target of quinolones and *parC* and *parE* are second (Heisig, 1996). Also, previous studies showing that mutations in the *parC* and/or *parE* are closely related to double mutations in the *gyrA* (Khodursky et al., 1995; Breines et al., 1997). Moreover, FQ-resistant *E. coli* isolates had mutations at codons 83 (Ser) and 87 (Asp) in *gyrA* and at codon 80 (Ser) in *parC* in the QRDRs, and the most common type of amino acid substitution were S83L and D87N in *gyrA* and S80I in *parC* as previous studies (Yang et al., 2012; Uchida et al., 2010; Yang et al., 2010). Also, MICs range of isolates with double mutations in *gyrA* were relatively higher than those of other isolates with single mutations in *gyrA*. Vila et al., (1994) reported that high-level resistance towards FQ is found if a second mutation accumulates in *gyrA*. Especially, FQ-resistant *E. coli* isolates which possessed double mutations in *gyrA* in combination with double mutations in *parC* or single mutations in both *parC* and *parE* were shown high levels of MICs range. These results were consistent with previous studies that the total number of point mutations in QRDR has been associated with the increased FQ-resistance levels (Liu et al., 2012; Hu et al. 2017).

In this study, 23 (21.7%) of the 106 FQ-resistant *E. coli* isolates detected PMQR genes. The prevalence of PMQR genes in FQ-resistant *E. coli* was considerably higher than that in a commercial broiler farm in Korea (17.8%) (Seo and Lee, 2020). These findings indicate that PMQR genes had already disseminated in broiler breeder and that the risk of PMQR spread in broiler production systems was considerable. Also, PMQR-positive *E. coli* isolates were carried 4 types of PMQR genes, *qnrS, qnrA, qnrB* and *aac(6’)-Ib-cr*. These PMQR variants have been previously detected in *E. coli* from livestock, including in healthy animals and retail meats in United States (Pereira et al., 2020), Taiwan (Kuo et al., 2009), Czech (Röderova et al., 2017), and China (Yu et al., 2015), as well as from commercial broiler farms and chicken meat in Korea (Seo and Lee, 2019, 2020).

Class 1 integrons can act as vectors that transfer and dissemination of antimicrobial resistance genes among bacteria and carry gene cassettes, which harbor antimicrobial resistance genes (Fluit and Schmitz, 2004). In this study, 16 (69.6%) PMQR-positive *E. coli* isolates contained class 1 integrons and 12 isolates have gene cassette that contains *aadA* or *dfra* or both genes. In previous studies, *aadA* and *dfra* gene were related resistance to antimicrobials such as aminoglycosides and trimethoprim and isolates harboring the *aadA* or *dfra* or both genes showed higher antimicrobial resistance rates (Seo and Lee, 2018). Therefore, integrons in PMQR-positive isolates from broiler breeder can have acquired the mobile genetic elements of antimicrobial resistance, which could become a serious public health concern. Also, 10 transconjugants identified in this study carried the same PMQR genes of the donor strains, demonstrating that PMQR-positive *E. coli* isolates may be transferred clonally to humans through contaminated food products of poultry origin, leading to treatment failure in humans.

Plasmids are important genetic elements responsible for the dissemination of antimicrobial resistance through horizontal gene transfer (Thomas and Nielsen, 2005; Yang et al., 2015). Especially, IncFIIA and IncFIIB replicons are reported as the most common types found in *E. coli* from humans and animals (Carattoli, 2009, Mitra et al., 2019, Son et al., 2019, Seo et al., 2020), and this was seen in this study. These plasmid replicons, which encode factors involved in iron uptake, toxin production, enzymes, and a variety of resistance genes, for example, PMQR genes, are widely spread in Enterobacteriaceae (Carattoli, 2009). Furthermore, other plasmid replicons such as IncFIC, IncFII, IncFrep, IncI1, IncB/O, and IncN identified in this study have also been previously reported (Carattoli, 2009, Pourel et al., 2011, Mitra et al., 2019, Son et al., 2019). Our results indicate that plasmid replicon types that are able to confer the antimicrobial resistance function to bacteria are common in PMQR-positive *E. coli* isolated from broiler breeder farms. Also, epidemiological relationships among the PMQR-positive isolates were examined by PFGE analysis in this study. Eight (34.8%) isolates included 3 PFGE patterns identified the same QRDR mutation, PMQR genes, plasmid replicon types, and
originated from the same PS farm, respectively. This results indicate the possibility that similar PFGE pattern isolates may contribute to clonal expansion and horizontal transmission as previously described (Tamang et al., 2014; Jo and woo 2016). This is the first study to investigate the prevalence and characteristics of FQ-resistant and PMQR-positive E. coli isolated from the broiler breeder in Korea; it supports that constant monitoring and studies at the broiler breeder level are required to prevent the pyramidal transmission of FQ-resistant E. coli.

ACKNOWLEDGMENTS

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Agriculture, Food and Rural Affairs Convergence Technologies Program for Educating Creative Global Leader, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA; 716002-7).

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Abdi-Hachesoo, B., K. Asasi, and H. Sharifiyazdi. 2017. Farm-level evaluation of enrofloxacin resistance in Escherichia coli isolated from broiler chickens during a rearing period. Comp. Clin. Pathol. 26:471–476.

Animal and Plant Quarantine Agency (APQA). 2017. National antimicrobial resistance monitoring program. Gimcheon, Republic of Korea.

Bai, H., J.-F. Du, M. Hu, J. Qi, Y.-N. Cai, W.-W. Niu, and Y.-Q. Liu. 2012. Analysis of mechanisms of resistance and tolerance of Escherichia coli to enrofloxacin. Ann. Microbiol. 62:293–298.

Breines, D. M., S. Ouabdesselam, E. Y. Ng, J. Tankovic, S. Shah, C. J. Souser, and D. C. Hooper. 1997. Quinolone resistance locus nfxD of Escherichia coli is a mutant allele of the parE gene encoding a subunit of topoisomerase IV. Antimicrob. Agents Chemother. 41:175–179.

Candrian, U., B. Furrer, C. Höfelein, R. Meyer, M. Jermini, and J. Lüthy. 1991. Detection of Escherichia coli and identification of entero-toxigenic strains by primer-directed enzymatic amplification of specific DNA sequences. Int. J. Food Microbiol. 12:339–351.

Carattoli, A. 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrob. Agents Chemother. 53:2227–2238.

Clinical and Laboratory Standards Institute. 2015. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals (VET01-S3). (3rd ed). Clinical and Laboratory Standards Institute, Wayne, PA.

Clinical and Laboratory Standards Institute. 2020. Performance Standards for Antimicrobial Susceptibility Testing. (30th ed. CLSI supplement M100). Clinical and Laboratory Standards Institute, Wayne, PA.

Dutta, S., Y. Kavamura, T. Ezaki, G. B. Nair, K.-I. Iida, and S.-I. Yoshida. 2005. Alteration in the GyrA subunit of DNA gyrase and the ParC subunit of topoisomerase IV in quinolone-resistant Shigella dysenteriae serotype 1 clinical isolates from Kolkata, India. Antimicrob. Agents Chemother. 49:1660–1661.

Fendukly, F., I. Karlsson, H. Hansson, G. Kronvall, and K. Dornbusch. 2003. Patterns of mutations in target genes in septicaemia isolates of Escherichia coli and Klebsiella pneumoniae with resistance or reduced susceptibility to ciprofloxacin. APMIS 111:857–866.

Fluit, A. C., and F. J. Schmitz. 2004. Resistance integrons and super-integrons. Clin. Microbiol. Infect. 10:272–288.

Heisig, P. 1996. Genetic evidence for a role of parC mutations in development of high-level fluoroquinolone resistance in Escherichia coli. Antimicrob. Agents Chemother. 40:879–885.

Hu, Y. S., S. Shin, Y. H. Park, and K. T. Park. 2017. Prevalence and mechanism of fluoroquinolone resistance in Escherichia coli isolated from Swine Feces in Korea. J. Food Prot. 80:1145–1151.

Jacoby, G. A. 2005. Mechanisms of resistance to quinolones. Clin. Infect. Dis. 41:S120–S126.

Jo, S. J., and G. J. Woo. 2016. Molecular characterization of plasmids encoding CTX-M beta-lactamases and their associated addiction systems circulating among Escherichia coli from retail chickens, chicken farms, and Slaughterhouses in Korea. J. Microbiol. Biotechnol. 26:270–276.

Johnson, T. J., Y. M. Wannemuehler, S. J. Johnson, C. M. Logue, D. G. White, C. Doetkott, and L. K. Nolan. 2007. Plasmid replicon typing of commensal and pathogenic Escherichia coli isolates. Appl. Environ. Microbiol. 73:1976–1983.

Kuo, H., C. Chou, C. Tu, S. Gong, C. Han, J. Liao, and S. Chang. 2009. Characterization of plasmid-mediated quinolone resistance by the qnr gene in Escherichia coli isolated from healthy chickens and pigs. Vet. Med. 54:473–482.

Khodursky, A. B., E. L. Zechiedrick, and N. R. Cozzarelli. 1995. Topoisomerase IV is a target of quinolones in Escherichia coli. Proc. Natl. Acad. Sci. USA 92:11801–11805.

Liu, B. T., X. P. Liao, S. S. Yang, X. M. Wang, L. L. Li, J. Sun, Y. R. Yang, L. X. Fang, L. Li, D. H. Zhao, and Y. H. Liu. 2012. Detection of mutations in the gyrA and parC genes in Escherichia coli isolates carrying plasmid-mediated quinolone resistance genes from diseased food-producing animals. J. Med. Microbiol. 61:1591–1599.

Liu, J. H., S. Y. Wei, J. Y. Ma, Z. L. Zeng, D. H. Liu, G. X. Yang, and Z. L. Chen. 2007. Detection and characterisation of CTX-M and CMY-2 beta-lactamases among Escherichia coli isolates from farm animals in Guangdong Province of China. Int. J. Antimicrob. Agents 29:576–581.

Magiorakos, A. P., A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber, and D. L. Monnet. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18:268–281.

Mitra, S., S. Mukherjee, S. Naha, P. Chattopadhyay, S. Dutta, and S. Basu. 2019. Evaluation of co-transfer of plasmid-mediated fluoroquinolone resistance genes and blanDM gene in Enterobacteriaceae causing neonatal septicaemia. Antimicrob. Resist. Infect. Control. 8:46.

Ng, W. L., K. M. R. Mulvey, I. Martin, G. A. Peters, and W. Johnson. 1999. Genetic Characterization of Antimicrobial Resistance in Canadian Isolates of Salmonella Serovar Typhimurium DT104. Antimicrob. Agents Chemother. 43:3018–3021.

Nishikawa, R., T. Murase, and H. Ozaki. 2019. Plasmid-mediated quinolone resistance in Escherichia coli isolates from commercial broiler chickens and selection of fluoroquinolone-resistant mutants. Poult. Sci. 98:5900–5907.

Patel, K., and J. L. Goldman. 2016. Safety concerns surrounding quinolone use in children. J. Clin. Pharmacol. 56:1060–1075.

Perera, R. V., C. Foditsch, J. D. Siler, S. C. Duli, C. Altier, A. Garzon, and L. D. Warnick. 2020. Genotypic antimicrobial resistance characterization of E. coli from dairy calves at high risk of respiratory disease administered enrofloxacin or tulathromycin. Sci. Rep. 10:19327.

Poirel, L., D. Dortet, S. Bernabeu, and P. Nordmann. 2011. Genetic features of blanDM-1-positive Enterobacteriaceae. Antimicrob. Agents Chemother. 55:5403–5407.
Röderova, M., D. Halova, I. Papousek, M. Dolejska, M. Masarikova, V. Hanulik, V. Pudova, P. Broz, M. Hrouzou-Sedlakova, P. Sauer, J. Bardon, A. Cizek, M. Kolar, and I. Literak. 2017. Characteristics of quinolone resistance in Escherichia coli isolates from humans, animals, and the environment in the Czech Republic. Front. Microbiol. 7:2147.

Sáenz, Y., L. Briñas, E. Domínguez, J. Ruiz, M. Zarazaga, J. Vila, and C. Torres. 2004. Mechanisms of resistance in multiple-antibiotic-resistant Escherichia coli strains of human, animal, and food origin. Antimicrob. Agents Chemother. 48:3996-4001.

Seo, K. W., and Y. M. Lee. 2018. Prevalence and characterization of β-lactamases genes and class 1 integrons in multidrug-resistant Escherichia coli isolates from chicken meat in Korea. Microb. Drug Resist. 24:1599–1606.

Seo, K. W., and Y. J. Lee. 2019. Characterization of plasmid-mediated quinolone resistance determinants in ciprofloxacin-resistant Escherichia coli from chicken meat produced by integrated broiler operations in Korea. Int. J. Food Microbiol. 307:108274.

Seo, K. W., and Y. J. Lee. 2020. Prevalence and Characterization of Plasmid-Mediated Quinolone Resistance Determinants qnr and aac(6’)-Ib-cr in Ciprofloxacin-Resistant Escherichia coli Isolates from Commercial Layer in Korea. J. Microbiol. Biotechnol. 30:1180–1183.

Seo, K. W., J. B. Shim, S. H. Son, E. B. Noh, S. Yoon, S. K. Lim, and Y. J. Lee. 2020. Impacts and characteristics of antimicrobial resistance of Escherichia coli isolates by administration of third-generation cephalosporins in layer hatcheries. Vet. Microbiol. 243:108643.

Son, S. H., K. W. Seo, Y. B. Kim, H. Y. Jeon, E. B. Noh, and Y. J. Lee. 2019. Molecular characterization of multidrug-resistant Escherichia coli Isolates from Edible Offal in Korea. J. Food Prot. 82:1183–1190.

Tamang, M. D., H. M. Nam, M. Gurung, G. C. Jang, S. R. Kim, S. C. Jung, Y. H. Park, and S. K. Lim. 2012. Molecular characterization of extended-spectrum-β-lactamase-producing and plasmid-mediated AmpC β-lactamase-producing Escherichia coli isolated from stray dogs in South Korea. Antimicrob. Agents Chemother. 56:2703–2712.

Tamang, M. D., M. Gurung, M. S. Kang, H. M. Nam, D. C. Moon, G. C. Jang, S. C. Jung, Y. H. Park, and S. K. Lim. 2014. Characterization of plasmids encoding CTX-M β-lactamase and their addiction systems in Escherichia coli isolates from animals. Vet. Microbiol. 174:456–462.

Taylor, N. M., R. H. Davies, A. Ridley, C. Clouting, A. D. Wales, and F. A. Clifton-Hadley. 2008. A survey of fluoroquinolone resistance in Escherichia coli and thermophilic Campylobacter spp. on poultry and pig farms in Great Britain. J. Appl. Microbiol. 105:1421–1431.

Thomas, C. M., and K. M. Nielsen. 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat. Rev. Microbiol. 3:711–721.

Uchida, Y., T. Mochimaru, Y. Morokuma, M. Kiyosuke, M. Fujise, F. Eto, Y. Harada, M. Kadowaki, N. Shimono, and D. Kang. 2010. Geographic distribution of fluoroquinolone-resistant Escherichia coli strains in Asia. Int. J. Antimicrob. Agents. 35:387–391.

United States Department of Agriculture (USDA). 2011. National Poultry Improvement Plan and Auxiliary Provisions. APHIS Publication, Riverdale, MD 91–55–088.

Vila, J., J. Ruiz, F. Marco, A. Barcelo, P. Goñi, E. Giralt, and T. Jimenez de Anta. 1994. Association between double mutation in gyrA gene of ciprofloxacin-resistant clinical isolates of Escherichia coli and MICs. Antimicrob. Agents Chemother. 38:2477–2479.

Waeyl, D., A. Hoszowski, M. Zajac, and K. Szulowska. 2013. Antimicrobial resistance in commensal Escherichia coli isolated from animals at slaughter. Front. Microbiol. 4:221.

WHO. 2017. Critically Important Antimicrobials for Human Medicine –5th rev. World Health Organization, Geneva, Switzerland.

Xu, Y., W. Yu, Q. Ma, and H. Zhou. 2015. Occurrence of (fluoro)quinolones and (fluoro)quinolone resistance in soil receiving swine manure for 11 years. Sci. Total Environ. 530-531:191–197.

Yang, H., Q. Yang, M. Chen, and H. Wang. 2008. High prevalence of plasmid-mediated quinolone resistance genes qnr and aac(6’)-Ib-cr in clinical isolates of Enterobacteriaceae from nine teaching hospitals in China. Antimicrob. Agents Chemother. 52:4268–4273.

Yang, H., S. Chen, D. G. White, S. Zhao, P. McDermott, R. Walker, and J. Meng. 2004. Characterization of multiple-antimicrobial-resistant Escherichia coli isolates from diseased chickens and swine in China. J. Clin. Microbiol. 42:3483–3489.

Yang, J., Y. Luo, J. Li, Y. Ma, C. Hu, S. Jin, L. Ye, and S. Cui. 2010. Characterization of clinical Escherichia coli isolates from China containing transferable quinolone resistance determinants. J. Antimicrob. Chemother. 65:453–459.

Yang, Q. E., J. Sun, L. Li, H. Deng, B. T. Liu, L. X. Fang, X. P. Liao, and Y. H. Liu. 2015. IncF plasmid diversity in multi-drug resistant Escherichia coli strains from animals in China. Front. Microbiol. 6:964.