Molecular characteristics of fluoroquinolone-resistant avian pathogenic Escherichia coli isolated from broiler chickens

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ABSTRACT Avian pathogenic Escherichia coli (APEC) is a major pathogen in the poultry industry worldwide including Korea. In this study, the phenotypic and genotypic characteristics of 33 fluoroquinolone (FQ)-resistant APEC isolates from broilers were analyzed. All FQ-resistant APEC isolates showed amino acid exchanges at both gyrA and parC and high minimal inhibitory concentrations for FQs. A total of 11 (33.3%) isolates were positive for the plasmid-mediated quinolone resistance (PMQR) genes, qnrA (8 isolates) and qnrS (3 isolates), and showed multidrug resistance. Among the 11 PMQR-positive isolates, 1 and 2 isolates carried blaCTX-M1 and blaCTX-M15, respectively, as extended-spectrum β-lactamase (ESBL) producers, and the non-ESBL gene, blaTEM-1, was found in 4 isolates. Among 3 aminoglycoside-resistant isolates, aac(3)-II was only detected in 1 isolate. All 8 APEC isolates with resistance to tetracycline carried the tetA gene. Overall, 6 of the 7 trimethoprim-sulfamethoxazole-resistant isolates carried the sul1 or sul2 genes, while only 2 of the 8 chloramphenicol-resistant isolates carried the catA1 gene. Among 3 aminoglycoside-resistant isolates, aac(3)-II was only detected in 1 isolate. All 8 APEC isolates with resistance to tetracycline carried the tetA gene. Overall, 6 of the 7 trimethoprim-sulfamethoxazole-resistant isolates carried the sul1 or sul2 genes, while only 2 of the 8 chloramphenicol-resistant isolates carried the catA1 gene. Although 9 isolates carried class I integrons, only 4 isolates carried the gene cassettes dfrA12-aadA2 (2 isolates), dfrA17-aadA5 (1 isolate), extX-psp-aadA2 (1 isolate), and dfrA27 (1 isolate). The most common plasmid replicon was FIB (8 isolates, 72.7%), followed by K/B (4 isolates, 36.4%). Antimicrobial resistance monitoring and molecular analysis of APEC should be performed continuously to surveil the transmission between poultry farms.

Key words: antimicrobial-resistant gene, APEC, broiler, multidrug resistance, plasmid-mediated quinolone resistance

INTRODUCTION

Colibacillosis caused by Escherichia coli (E. coli) in mammals is most often a primary enteric or urinary tract disease, whereas colibacillosis in poultry is typically a localized or systemic disease occurring secondarily when host defenses have been impaired or overwhelmed by virulent E. coli strains (Jahantigh and Dizaji, 2015). Avian pathogenic E. coli (APEC) is a major pathogen in the poultry industry worldwide and often causes severe colibacillosis after respiratory stress from infections with Mycoplasmas or respiratory viral agents (Matthijs et al., 2003). In Korea, many poultry flocks also suffer from infection with APEC (Kim et al., 2007, 2009; Oh et al., 2011). Therefore, the use of antimicrobial drugs such as β-lactams, aminoglycosides, and fluoroquinolones (FQs) has remained the primary option for controlling colibacillosis. FQs are broad-spectrum antibacterial agents and exert their effects by binding to and inhibiting bacterial DNA gyrase. Since enrofloxacin (ENR) have been introduced to the poultry industry in Korea in 1987, they have been widely used throughout the country for mass medication in farms. Approximately 50 tons of FQs are sold every year for animal production, including poultry in Korea (Kim et al., 2018). However, the continuous use of FQs in poultry production has resulted in the emergence and maintenance of fluoroquinolone (FQ)-resistant APEC (Kim et al., 2009). The World Health Organization has classified quinolones as “critically important antimicrobials for human medicine” because FQ-resistant microorganisms are a

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serious global public and animal health problem (WHO, 2017). Several researchers have reported that most cases of FQ resistance in human zoonotic infections may be attributed to farm animal antimicrobial use (Endtz et al., 1991; Chiu et al., 2002). Evidence for resistance transmission from farm animals to humans is particularly strong in the use of the antimicrobials in poultry (Johnson et al., 2006). In Korea, the mass medication of poultry with ENR is still permitted, and the sale volume of ENR is the highest among all antimicrobials used to treat poultry (APQA, 2017). Although previous studies have found that FQ treatment against colibacillosis in chicken could result in FQ-resistant APEC, a comprehensive evaluation of the virulence and resistance of isolates has not been fully performed in Korea. Therefore, this study was conducted to determine the phenotypic and genotypic characteristics of FQ-resistant APEC.

**MATERIALS AND METHODS**

**Sampling**

Liver swab samples were collected from 60 broiler farms suffering from colibacillosis nationwide in 2018. The swabs were placed in transport medium (Noble Bio, Hwaseong, Korea) and sent to the laboratory in a cooler. All specimens were inoculated into 10 mL of modified E. coli broth with Novobiocin (Merck, Darmstadt, Germany) within 24 h of collection.

**Bacterial Isolates**

The enriched modified E. coli was streaked onto MacConkey agar (BD Biosciences, Sparks, MD) containing 4 μg/mL of ciprofloxacin (CIP; Sigma-Aldrich, St. Louis, MO). Subsequently, suspected E. coli colonies were identified by PCR as previously described (Candrian et al., 1991). Confirmed E. coli were also analyzed for 5 genes (iroN, ompT, hlyF, iss, and iutA) as the minimal predictors of APEC virulence described by Johnson et al. (2008). If isolates from the same farm showed the same antimicrobial susceptibility patterns, one isolate was randomly selected. A total of 33 FQ-resistant APEC isolates were included in this study.

**Antimicrobial Susceptibility Test**

The disk diffusion method was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). The 16 antimicrobial disks (BD Biosciences) used in this study were amoxicillin-clavulanate (20/10 μg), ampicillin (10 μg), cefazolin (30 μg), cefepime (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefuroxime (30 μg), cephalexin (30 μg), cephalothin (30 μg), chloramphenicol (C, 30 μg), CIP (5 μg), gentamicin (10 μg), imipenem (10 μg), tetracycline (TE, 30 μg), and trimethoprim-sulfamethoxazole (1.25/23.75 μg). Multidrug resistance (MDR) was defined as acquired resistance to at least one agent in 3 or more antimicrobial classes (Magiorakos et al., 2012). The minimum inhibitory concentration (MIC) for CIP, ENR, and norfloxacin (NOR) was determined by standard agar dilution methods using the Mueller-Hinton agar (BD Biosciences) method according to the guidelines of the CLSI (CLSI, 2017). The breakpoints of CIP and NOR were determined according to the guidelines of the CLSI (CLSI, 2017), and the breakpoint of ENR was determined according to the guidelines of the CLSI (2002). E. coli ATCC 25922 was used as a quality control strain.

**Serogrouping**

O-serogroups were determined by multiplex PCR using 162 primer pairs including O1 to O187 as described by Iguchi et al. (2015).

**Analysis of Quinolone Resistance-Determining Regions**

PCR was performed to amplify the gyrA and parC of the quinolone resistance-determining region to identify mutations in 33 FQ-resistant APEC isolates using primers and conditions described previously (Pons et al., 2014). The PCR products were purified using GFX PCR DNA and the Gel Band Purification Kit (Amersham Biosciences, Freiburg, Germany) and sequenced using an automatic sequencer (Cosmogene-tech, Seoul, Korea). The sequences were compared with those in the GenBank nucleotide database using the Basic Local Alignment Search Tool program available through the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST).

**Molecular Analysis**

The detection of integrons, antimicrobial resistance genes, and gene cassettes was performed by PCR using the primers described in Table 1. All FQ-resistant APEC isolates were tested for resistance genes related to β-lactam antimicrobials (blaTEM, blaSHV, blaOXA, and blactx-M families), quinolones (qnrA, qnrB, qnrD, qnrS, and qepA), TE (tetA, tetB, and tetC), C (cmlA and catA), sulfonamides (sul1 and sul2), and aminoglycosides (aac (3)-II, ant (2’)-I, and aac (6’)-Ib). Class 1 and 2 integrons (intI1 and intI2) were also investigated.

The presence of gene cassettes in integron-positive isolates was determined. The purification and sequencing of PCR products were performed as described previously. Gene cassette homology was performed by Basic Local Alignment Search Tool analysis (www.ncbi.nlm.nih.gov/BLAST).

**Plasmid Replicon Typing**

All FQ-resistant APEC isolates were screened for 18 plasmid replicons using a PCR-based typing method with 3 multiplex panels as previously described (Johnson et al., 2007).
Table 1. Primer sequences used for the amplification.

| Primer Sequence (5’ → 3’) | Size (bp) | Reference |
|----------------------------|-----------|-----------|
| **E. coli identification** |           |           |
| *E. coli*                  |           |           |
| F: GACCTCGGTTTAGTTCACAGA   | 585       | Candrian et al. 1991 |
| R: CACACGCTGACGCTGACCA     |           |           |
| **Quinolone resistance determining region (QRDR)** |       |           |
| *gyrA*                     |           |           |
| F: AAATCTGCCCGTGTCGTTGGT   | 343       | Pons et al. 2014 |
| R: GCCATACCTACGGCGATACC    |           |           |
| *parC*                     |           |           |
| F: AAACCTGTTCAGCGCGCATT    | 395       | Pons et al. 2014 |
| R: GTGTTGCCGTTAAAGCAA      |           |           |
| **Plasmid-mediated quinolone** |       |           |
| *qnrA*                     |           |           |
| F: TCAGCAAGAGGATTCTCCA     | 627       | Wang et al. 2003 |
| R: GGCACGACTATATCTCCA      |           |           |
| *qnrB*                     |           |           |
| F: CGCCTGACGGCGACTGAAT     | 515       | Jiang et al. 2008 |
| R: TGACCAACGATGCCCTGAGT    |           |           |
| *qnrD*                     |           |           |
| F: CGAGATCTAAATTTACGGGGAATA | 582     | Cavaco et al. 2009 |
| R: AAACAGCTGAAGGCGCTG      |           |           |
| *qnrS*                     |           |           |
| F: ACCTTCACCGCTTCAATT      | 571       | Jiang et al. 2008 |
| R: CCAATGTCCTGAGAATCATCAGT|           |           |
| *qepA*                     |           |           |
| F: CGTGTGCTGAGTTCCTC       | 403       | Minarini et al. 2008 |
| R: CTCCAGGTACCTGCGTCTAG    |           |           |
| **β-lactamases**           |           |           |
| *TEM*                      |           |           |
| F: CATTTCCTGCTGCCCTTATTC   | 800       | Dallenne et al. 2010 |
| R: CGTTCTACATCAGTTGCTGAC   |           |           |
| *SHV*                      |           |           |
| F: CACTCAAGGATGATTGTTG     | 885       | Briñas et al. 2002 |
| R: TTAGCCGTGGCAGTGCCTG     |           |           |
| *OXA*                      |           |           |
| F: TTTAAGCCAAAGACAGCATG    | 702       | Briñas et al. 2002 |
| R: TCCAGACCAAGGCGGCTGGTT   |           |           |
| **CTX-M group I**          |           |           |
| F: GACGATGCATCTGCGTACG     | 499       | Pitout et al. 2004 |
| R: AGCCGGCAGCCTAATACA      |           |           |
| **CTX-M group II**         |           |           |
| F: GCGACCTGTTAATCTACATCC   | 351       | Pitout et al. 2004 |
| R: CGATGTTATCGCCCTAAAGCC   |           |           |
| **CTX-M group III**        |           |           |
| F: CGGTTCGACATGCGACGCC     | 307       | Pitout et al. 2004 |
| R: GCTCAGTACGATCGCACCC     |           |           |
| **CTX-M group IV**         |           |           |
| F: GCTGGAAGAAAGGACGCGAG    | 474       | Pitout et al. 2004 |
| R: GTAAGCTGACCGCAACGTCG    |           |           |
| **Aminoglycoside-modifying enzymes (AMEs)** |     |           |
| *aac(6')-Ib*              |           |           |
| F: TGCCCATTGCAGTCGCTGTATG  | 509       | Jiang et al. 2008 |
| R: TTAGGGCATCAGTCGGTTTC    |           |           |
| *aac(3)-II*                |           |           |
| F: TGAACCTGCGAGGACCTCTC    | 369       | Sandvang and Aarestrup. 2000 |
| R: GTGACCAAGTACGACTGAG     |           |           |
| *ant(2')-I*                |           |           |
| F: GGCGCCGTGCATAGGAGTTT    | 740       | Sandvang and Aarestrup. 2000 |
| R: TATCGGGACCTGAAAGGCCG    |           |           |
| **Tetracyclines**          |           |           |
| *tetA*                     |           |           |
| F: GTATTCTGAGCAGCTGTCGC    | 956       | Sengeløv et al. 2003 |
| R: CTGCCCTGAGCACAACATTGCTT|           |           |
| *tetB*                     |           |           |
| F: CTCAGTATTTCAAGGCTTGT    | 414       | Sengeløv et al. 2003 |
| R: ACTCCCGCTGGCTTGAGGGG    |           |           |
| *tetC*                     |           |           |
| F: CCTCTTCTGCGGAGATATCGCC  | 505       | Sengeløv et al. 2003 |
| R: GTTGAGAGCTCTCAAGGCC      |           |           |
| **Sulfonamide**            |           |           |
| *sul1*                     |           |           |
| F: CTTGCTGAGGACGCGCGCGGC   | 433       | Sandvang et al. 1998 |
| R: GCAAGCGGGAACCGCAGGCC    |           |           |
| *sul2*                     |           |           |
| F: CGCGCATGCTCAACATAACC    | 722       | Maynard et al. 2003 |
| R: GTGTGCGGATGAACTGAC      |           |           |
| **Chloramphenicol**        |           |           |
| *catA1*                    |           |           |
| F: AGTGTGCTCAATGTAATCAAACC| 547       | Van et al. 2008 |
| R: TTGTAATATTACAAGCATTCTGC|           |           |
| *cmlA*                     |           |           |
| F: CGCGCATGCTGGTGTGGTATAC | 698       | Van et al. 2008 |
| R: CACCTTCTGCGTCCCATCTAG  |           |           |
| **Integrons and cassettes**|           |           |
| Class 1 integron           |           |           |
| F: GCCGTGGTCTTCTTCTACGG    | 558       | Ng et al. 1999 |
| R: GATGGGCGCTCTTGACTACGG   |           |           |
| Class 1 cassettes          |           |           |
| F: GCCATCAGACGAGCGAAG      | variable  | Ng et al. 1999 |
| R: AAGCAGACTTGGACCTGA      |           |           |

(continued on next page)
Transconjugation

The transfer of plasmid-mediated quinolone resistance (PMQR) genes was performed by conjugation experiments using the broth mating method with sodium azide-resistant *E. coli* J53 as a recipient (Tamang et al., 2012). Transconjugants were selected on MacConkey agar (BD Biosciences) plates with

| Primer             | Sequence (5' → 3’)                          | Size (bp) | Reference          |
|-------------------|---------------------------------------------|-----------|--------------------|
| Class 2 integron  | F: CACGGATATGCAGACAAAAGGT                   | 788       | Sáenz et al. 2004  |
|                   | R: GTAGCAAACGAGTAGCGAACAAATG                |           |                    |
| Class 2 cassettes | F: CGGGATCCCGGACGGCATG                     | variable  | Sáenz et al. 2004  |
|                   | CACGATTGTA                                   |           |                    |
|                   | R: GATGCCATCGCAAGTGACGAG                    |           |                    |

Abbreviations: CTX, cefotaxime; OXA, oxacillinase.

**Figure 1.** Antimicrobial resistance spectrum (A) and classes (B) in 33 fluoroquinolone-resistant avian pathogenic *E. coli* isolates. AMGs, aminoglycosides; BL/BLICs, β-lactam/β-lactamase inhibitor combinations; CARs, carbapenems; CEPs, cephems; FPIs, folate pathway inhibitors; PCNs, penicillins; PHs, phenicols; TETs, tetracyclines.
sodium azide (100 μg/mL; Sigma-Aldrich, ST Louis, MO) and ampicillin or TE (100 μg/mL; Sigma-Aldrich). Transferability was confirmed by antimicrobial susceptibility tests and PCR for molecular analysis as described previously.

**RESULTS**

**Antimicrobial Resistance**

The antimicrobial resistance analysis is shown in Figure 1. All FQ-resistant APEC isolates showed the highest resistance to penicillins (90.9%), followed by TE (78.8%), phenicols (66.7%), folate pathway inhibitors (57.6%), cephalosporins (45.5%), aminoglycosides (12.1%), and β-lactam/β-lactamase inhibitor combinations (6.1%). A total of 30 (90.9%) APEC isolates were identified as having MDR. The rate of resistance to 3 antimicrobial classes was the highest at 30.3%, and one (3.0%) FQ-resistant APEC isolate showed resistance to 6 classes.

**Characteristics of FQ-Resistant APEC**

The molecular characteristics of 33 FQ-resistant APEC isolates are shown in Table 2. Among the isolates, 30 isolates were classified into 18 O-serogroups, and 3 isolates were ungrouped. The most common serogroup was O78 (5 isolates, 15.2%). All FQ-resistant APEC isolates showed amino acid exchanges at both gyrA and parC, and the MIC ranges for CIP, ENR, and NOR were 4 to 128 μg/mL, 8 to 128 μg/mL, and 8 to >512 μg/mL, respectively. A total of 11 (33.3%) isolates were positive for the PMQR genes qnrA (8 isolates) and qnrS (3 isolates). However, only one of 7 isolates, which showed the highest MICs for CIP (≥64 μg/mL), ENR (128 μg/mL), and NOR (≥256 μg/mL), carried the PMQR gene qnrA.

**Characterization of PMQR-Positive FQ-Resistant APEC**

The phenotypic and genotypic characteristics of 11 PMQR-positive FQ-resistant APEC isolates are shown in Table 3. All PMQR-positive isolates showed MDR with resistance to 3-11 antimicrobials. Five isolates were identified as β-lactamase-producing APEC. As extended-spectrum β-lactamase producers, one and 2 isolates carried blaCTX-1 and blaCTX-15, respectively. The non–extended-spectrum β-lactamase gene, blaTEM-1, was found in 4 isolates. Of the 5 β-lactamase–producing

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**Table 2.** Amino acid changes within QRDRs and prevalence of PMQR genes in 33 fluoroquinolone-resistant avian pathogenic *E. coli* isolates.

| O Serotype | PMQR genes | Amino acid change | MIC (μg/mL) | No. of isolates included |
|------------|------------|------------------|-------------|-------------------------|
|            | (gyrA)     | (parC)           | CIP | ENR | Nor |            |
| O2         | -          | S83 L/D87 | S80 R | 4   | 8   | 8   | 3          |
| O3         | -          | S83 L/D87 N | S80 I | 8   | 16  | 16  | 1          |
| O3         | -          | S83 L/D87 N | S80 R | 4   | 16  | 16  | 1          |
| O3         | -          | S83 L/D87 N | S80 I | 8   | 32  | 16  | 1          |
| O9         | qnrA       | S83 L/D87 N | S80 I | 8   | 32  | 16  | 1          |
| O9         | qnrA       | S83 L/D87 N | S80 I | 16  | 32  | 32  | 1          |
| O45        | -          | S83 L/D87 Y | S80 I | 16  | 128 | 64  | 1          |
| O45        | -          | S83 L/D87 N | S80 I/E84 G | 64 | 128 | >512 | 1          |
| O78        | qnrA       | S83 L/D87 N | S80 I | 8   | 32  | 16  | 1          |
| O78        | -          | S83 L/D87 N | S80 I | 16  | 32  | 64  | 1          |
| O78        | -          | S83 L/D87 N | S80 I | 8   | 32  | 16  | 1          |
| O78        | -          | S83 L/D87 N | S80 I/E84 A | 64 | 128 | >512 | 1          |
| O78        | -          | S83 L/D87 N | S80 I/E84 G | 64 | 128 | >512 | 1          |
| O86        | -          | S83 L/D87 N | S80 I | 128 | 128 | 256 | 1          |
| O86        | -          | S83 L/D87 N | S80 I | 64  | 128 | >512 | 1          |
| O88        | qnrA       | S83 L/D87 N | S80 I | 8   | 32  | 16  | 1          |
| O88        | -          | S83 L/D87 N | S80 I | 8   | 16  | 16  | 1          |
| O99        | -          | S83 L/D87 Y | S80 R | 8   | 32  | 16  | 1          |
| O104       | -          | S83 L/D87 N | S80 I | 8   | 32  | 16  | 1          |
| O111       | -          | S83 L/D87 N | S80 I | 8   | 32  | 16  | 1          |
| O115       | qnrA       | S83 L/D87 E | S80 I | 8   | 32  | 16  | 1          |
| O128       | -          | S83 L/D87 Y | S80 I | 16  | 64  | 64  | 1          |
| O128       | qnrS       | S83 L/D87 N | S80 I | 16  | 32  | 128 | 1          |
| O133       | -          | S83 L/D87 N | S80 R | 8   | 16  | 16  | 1          |
| O141       | qnrS       | S83 L/D87 N | S80 I | 16  | 32  | 16  | 1          |
| O148       | -          | S83 L/D87 N | S80 I | 32  | 64  | 64  | 1          |
| O166       | -          | S83 L/D87 N | S80 I | 8   | 32  | 16  | 1          |
| O9g82qnrA  | S83 L/D87 N | S80 I | 8   | 32  | 16  | 1          |
| O9g8qnrS   | S83 L/D87 N | S80 I | 16  | 32  | 16  | 1          |
| ONTg8      | qnrA       | S83 L/D87 N | S80 I | 64  | 128 | 256 | 1          |
| ONT        | qnrA       | S83 L/D87 N | S80 I | 16  | 32  | 64  | 1          |
| ONT        | -          | S83 L/D87 N | S80 I | 128 | 128 | >512 | 1          |

Abbreviations: CIP, ciprofloxacin; ENR, enrofloxacin; MIC, minimum inhibitory concentration; NOR, norfloxacin; PMQR, plasmid-mediated quinolone resistance; QRDR, quinolone resistance-determining regions.

1Not detected.
2O107 or O117.
3Untyped.
Table 3. Phenotypes and genotypes of 11 PMQR-positive avian pathogenic E. coli isolates.

| Strain no. | PMQR genes | Resistance phenotypes | Resistance genes | Integron and gene cassettes | Plasmid replicon type |
|-----------|------------|-----------------------|------------------|-----------------------------|-----------------------|
| CC-22-10  | O         | TEM-1, CTX-1           | tetA             |                             | FIB                   |
| CC-32-20  | O         | AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, C, CTX, CXM, CZ, GM | tetA |                             | O88                   |
| CC-32-48  | O         | AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, C, CTX, CXM, CZ, GM | tetA, dfr, ada, A5 |                             | FIB                   |
| CC-32-48  | O         | AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, C, CTX, CXM, CZ, GM | tetA, dfr, ada, A2 |                             | FIB                   |
| CC-32-48  | O         | AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, C, CTX, CXM, CZ, GM | tetA, dfr, ada, A2 |                             | FIB                   |
| CC-32-48  | O         | AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, C, CTX, CXM, CZ, GM | tetA, dfr, ada, A2 |                             | FIB                   |
| CC-32-48  | O         | AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, C, CTX, CXM, CZ, GM | tetA, dfr, ada, A2 |                             | FIB                   |
| CC-32-48  | O         | AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, C, CTX, CXM, CZ, GM | tetA, dfr, ada, A2 |                             | FIB                   |
| CC-32-48  | O         | AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, C, CTX, CXM, CZ, GM | tetA, dfr, ada, A2 |                             | FIB                   |
| CC-32-48  | O         | AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, C, CTX, CXM, CZ, GM | tetA, dfr, ada, A2 |                             | FIB                   |
| CC-32-48  | O         | AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, C, CTX, CXM, CZ, GM | tetA, dfr, ada, A2 |                             | FIB                   |

**DISCUSSION**

APEC is associated with extraintestinal infections in poultry and is considered one of the main causes of mortality and morbidity, resulting in heavy economic losses in the industry worldwide including Korea (Kim et al., 2007; Oh et al., 2011; Varga et al., 2018). Antimicrobials play a key role in treating and preventing infectious diseases in livestock including poultry (Yang et al., 2004; Li et al., 2015). In particular, FQs are broad-spectrum synthetic drugs used extensively for the control of bacterial infections in Korea (APQA, 2017). Resistance to FQs has emerged after their widespread use; thus, the probability of treatment failure may be increased (Li et al., 2015). Moreover, the prevalence and dissemination of resistance of FQs in APEC have increased significantly in recent years because FQ-resistant E. coli often exhibits a multidrug-resistant phenotype (Kim et al., 2007; Seo and Lee, 2018; 2019). In this study, 33 FQ-resistant APEC isolates showed coresistance to penicillins (90.9%), TEs (78.8%), phenicols (66.7%), and folate pathway inhibitors (57.6%), and 30 (90.1%) isolates expressed a typical MDR phenotype with antimicrobial resistance to 3-7 antimicrobial classes including FQs. These results are consistent with those of recent studies showing high MDR rates among FQ-resistant E. coli (Li et al., 2015; Seo and Lee, 2019).

Bacterial resistance to FQs is caused by mutations in the quinolone resistance-determining regions. In gram-negative bacteria including E. coli, gyrA is the primary target and commonly exhibits substitutions at amino acid residues 83 and 87. Substitutions at amino acid residues 80 and 84 in the parC subunit of topoisomerase IV are less common. In this study, all FQ-resistant APEC isolates had mutations in both the gyrA and parC genes.
In particular, 32 (97.0%) isolates had double point mutations in \(gyrA\), and the most common mutation was S83 L/D87 N (24 isolates). Also, previous studies report that S83 L and D87 N in \(gyrA\) and S80I in \(parC\) were the most common type of amino acid substitution in \(E. coli\) (Yang et al., 2004; Uchida et al., 2010; Liu et al., 2012). However, regardless of the various double amino acid substitutions in \(gyrA\), there was no significant difference in the MICs. Only 3 isolates had double point mutations in the \(parC\) gene, which included S80I/E84 G (2 isolates) and S80I/E84 A (1 isolate). But these 3 isolates possessed both double mutation at \(gyrA\) and \(parC\) and showed the highest MICs for CIP (64 \(\mu g/mL\)), ENR (128 \(\mu g/mL\)), and NOR (>512 \(\mu g/mL\)).

In this study, 11 FQ-resistant APEC isolates carried 2 types of PMQR genes, \(qnrS\) (3 isolates) and \(qnrA\) (8 isolates). This result is consistent with recent studies of APEC isolates from Egypt, Taiwan, and South Korea (Ahmed et al., 2013; Yeh et al., 2017; Seo and Lee, 2019). The PMQR genes may contribute to the increased prevalence of resistant mutants by conferring a low resistance level in a population (Varela et al., 2015). In this study, although 7 isolates showed the highest MICs for CIP (≥64 \(\mu g/mL\)), ENR (128 \(\mu g/mL\)), and NOR (≥256 \(\mu g/mL\)), only one isolate carried the PMQR gene, \(qnrA\). However, 11 PMQR-positive APEC isolates carried a variety of antimicrobial resistance genes such as \(bla_{CTX-M1}\), \(bla_{CTX-M15}\), \(bla_{TEM1}\), \(aac(3)-II\), \(tetA\) sul1, sul2, and \(cat\)AA1 and harbored mobile elements such as integrons and gene cassettes at the same time. The rise of antimicrobial resistance is thought to be closely associated with the widespread transfer of resistance genes between bacterial species. CTX-M-type \(\beta\)-lactamase genes hydrolyze the characteristic \(\beta\)-lactam ring and confer resistance to most \(\beta\)-lactam antimicrobials, including cephalosporins (Paterson and Bonomo., 2005). The \(bla_{TEM1}\) gene code for narrow-spectrum \(\beta\)-lactamase that can inactivate penicillins and aminopenicillins (Poirel et al., 2018). The prevalence of the PMQR genes in poultry varies in Korea (Oh et al., 2016; Seo and Lee, 2019); however, the PMQR genes in \(\beta\)-lactamase-producing \(E. coli\) were detected at high levels (Seo and Lee, 2019). The presence of the PMQR genes may be significantly associated with the \(\beta\)-lactamase gene, perhaps due to common carriage on a plasmid in \(Enterobacteriaceae\) (Xue et al., 2017).

In this study, 6 and one of 11 PMQR-positive APEC isolates contained class 1 and 2 integrons, respectively. Five isolates also contained at least one more cassette. Although \(dfrA\)-aadA was the dominant gene cassette array in this study and has been identified in \(E. coli\) from the poultry industry (Kim et al., 2007; Dessie et al., 2013; Seo and Lee, 2018), this is the first report of this cassette in APEC isolates in Korea.

Plasmids are extrachromosomal genetic elements that act as excellent delivery vectors for the dissemination of antimicrobial resistance through horizontal gene transfer (Yang et al., 2015; Son et al., 2019). In our study, most isolates (90.1%) among the PMQR-positive APEC isolates harbored IncF plasmids including FIA, FIB, and FIC. Wu et al. (2010) and Yang et al. (2015) have suggested that IncF plasmids may be associated with a wide range of genes conferring resistance to important classes of antimicrobials including quinolones, \(\beta\)-lactams, TEs, sulfonamides, chloramphenicol, and aminoglycosides.

This study investigated the molecular characteristics of FQ-resistant APEC from broiler chickens. Almost all FQ-resistant APEC showed MDR phenotype, and the most prevalent of the mutations were double point mutations in \(gyrA\) and single mutation in \(parC\). FQ-resistant APEC with PMQR genes carried various antimicrobial genes and harbored mobile elements and plasmid replications. The overuse of various antimicrobials in poultry production may have served as a major selection pressure for the horizontal transfer of resistance elements. Therefore, antimicrobial resistance monitoring and molecular analysis of APEC should be performed continuously to surveil the transmission between poultry farms.

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