MICROBIOLOGY AND FOOD SAFETY

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 $bla_{\text{CTX-1}}$ and $bla_{\text{CTX-15}}$, respectively, as extended-spectrum β-lactamase (**ESBL**) producers, and the non-ESBL gene, $bla_{\text{TEM-1}}$, 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. Although 9 isolates carried class I integrons, only 4 isolates carried the gene cassettes *dfr*A12-*aad*A2 (2 isolates), *dfr*A17-*aad*A5 (1 isolate), *ext*X-*psp*-*aad*A2 (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

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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 flouoroquinolone (**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), cefoxitin (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 (Cosmogenetech, 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 (bla_{TEM}, bla_{SHV}, bla_{OXA}, and $bla_{\text{CTX-M}}$ families), quinolones (*qnrA*, *qnrB*, *qnrD*, *qnrS*, and *qepA*), TE (*tetA*, *tetB*, and *tetC*), C (*cmlA* and *catA1*), sulfonamides (*sul1* and *sul2*), and aminoglycosides (*aac (3)-II*, *ant (2″)-I*, and *aac (6′)-Ib*). Class 1 and 2 integrons (*intl1* and *intl2*) 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 R: CACACGCTGACGCTGACCA	585	Candrian et al. 1991
Quinolone resistance determining region (QRDR)			
gyrA	F: AAATCTGCCCGTGTCGTTGGT R: GCCATACCTACGGCGATACC	343	Pons et al. 2014
parC	F: AAACCTGTTCAGCGCCGCATT R: GTGGTGCCGTTAAGCAAA	395	Pons et al. 2014
Plasmid-mediated quinolone			
qnrA	F: TCAGCAAGAGGATTTCTCA R: GGCAGCACTATTACTCCCA	627	Wang et al. 2003
qnrB	F: CGACCTGAGCGGCACTGAAT R: TGAGCAACGATGCCTGGTAG	515	Jiang et al. 2008
qnrD	F: CGAGATCAATTTACGGGGAATA R: AACAAGCTGAAGCGCCTG	582	Cavaco et al. 2009
qnrS	F: ACCTTCACCGCTTGCACATT R: CCAGTGCTTCGAGAATCAGT	571	Jiang et al. 2008
qepA	F: CGTGTTGCTGGAGTTCTTC R: CTGCAGGTACTGCGTCATG	403	Minarini et al. 2008
β-lactamases			
TEM	F: CATTTCCGTGTCGCCCTTATTC R: CGTTCATCCATAGTTGCCTGAC	800	Dallenne et al. 2010
SHV	F: CACTCAAGGATGTATTGTG R: TTAGCGTTGCCAGTGCTCG	885	Briñas et al. 2002
OXA	F: TTCAAGCCAAAGGCACGATAG R: TCCGAGTTGACTGCCGGGTTG	702	Briñas et al. 2002
CTX-M group I	F: GACGATGTCACTGGCTGAGC R: AGCCGCCGACGCTAATACA	499	Pitout et al. 2004
CTX-M group II	F: GCGACCTGGTTAACTACAATCC R: CGGTAGTATTGCCCTTAAGCC	351	Pitout et al. 2004
CTX-M group III	F: CGCTTTGCCATGTGCAGCACC R: GCTCAGTACGATCGAGCC	307	Pitout et al. 2004
CTX-M group IV	F: GCTGGAGAAAAGCAGCGGAG R: GTAAGCTGACGCAACGTCTG	474	Pitout et al. 2004
Aminoglycoside-modifying enzymes (AMEs)			
aac(6′)-Ib	F: TGACCTTGCGATGCTCTATG R: TTAGGCATCACTGCGTGTTC	509	Jiang et al. 2008
aac(3)-II	F: TGAAACGCTGACGGAGCCTC R: GTCGAACAGGTAGCACTGAG	369	Sandvang and Aarestrup. 2000
ant(2″)-I	F: GGGCGCGTCATGGAGGAGTT R: TATCGCGACCTGAAAGCGGC	740	Sandvang and Aarestrup. 2000
Tetracyclines			
tetA	F: GTAATTCTGAGCACTGTCGC R: CTGCCTGGACAACATTGCTT	956	Sengeløv et al. 2003
tetB	F: CTCAGTATTCCAAGCCTTTG R: ACTCCCCTGAGCTTGAGGGG	414	Sengeløv et al. 2003
tetC	F: CCTCTTGCGGGATATCGTCC R: GGTTGAAGGCTCTCAAGGGC	505	Sengeløv et al. 2003
Sulfonamide			
sul1	F: CTTCGATGAGAGCCGGCGGC R: GCAAGGCGGAAACCCGCGCC	433	Sandvang et al. 1998
sul2	F: CGGCATCGTCAACATAACC R: GTGTGCGGATGAAGTCAG	722	Maynard et al. 2003
Chloramphenicol			
catA1	F: AGTTGCTCAATGTACCTATAACC R: TTGTAATTCATTAAGCATTCTGCC	547	Van et al. 2008
cmlA	F: CCGCCACGGTGTTGTTGTTATC R: CACCTTGCCTGCCCATCATTAG	698	Van et al. 2008
Integrons and cassettes			
Class 1 integron	F: GCCTTGCTGTTCTTCTACGG R: GATGCCTGCTTGTTCTACGG	558	Ng et al. 1999
Class 1 cassettes	F: GGCATCCAAGCAGCAAG R: AAGCAGACTTGACCTGA	variable	Ng et al. 1999

(continued on next page)

Table 1. (*continued*)

Primer	Sequence (5′ → 3′)	Size (bp)	Reference
Class 2 integron	F: CACGGATATGCGACAAAAAGGT R: GTAGCAAACGAGTGACGAAATG	788	Sáenz et al. 2004
Class 2 cassettes	F: CGGGATCCCGGACGGCATG CACGATTTGTA R: GATGCCATCGCAAGTACGAG	variable	Sáenz et al. 2004

Abbreviations: CTX, cefotaxime; OXA, oxacillinase.

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

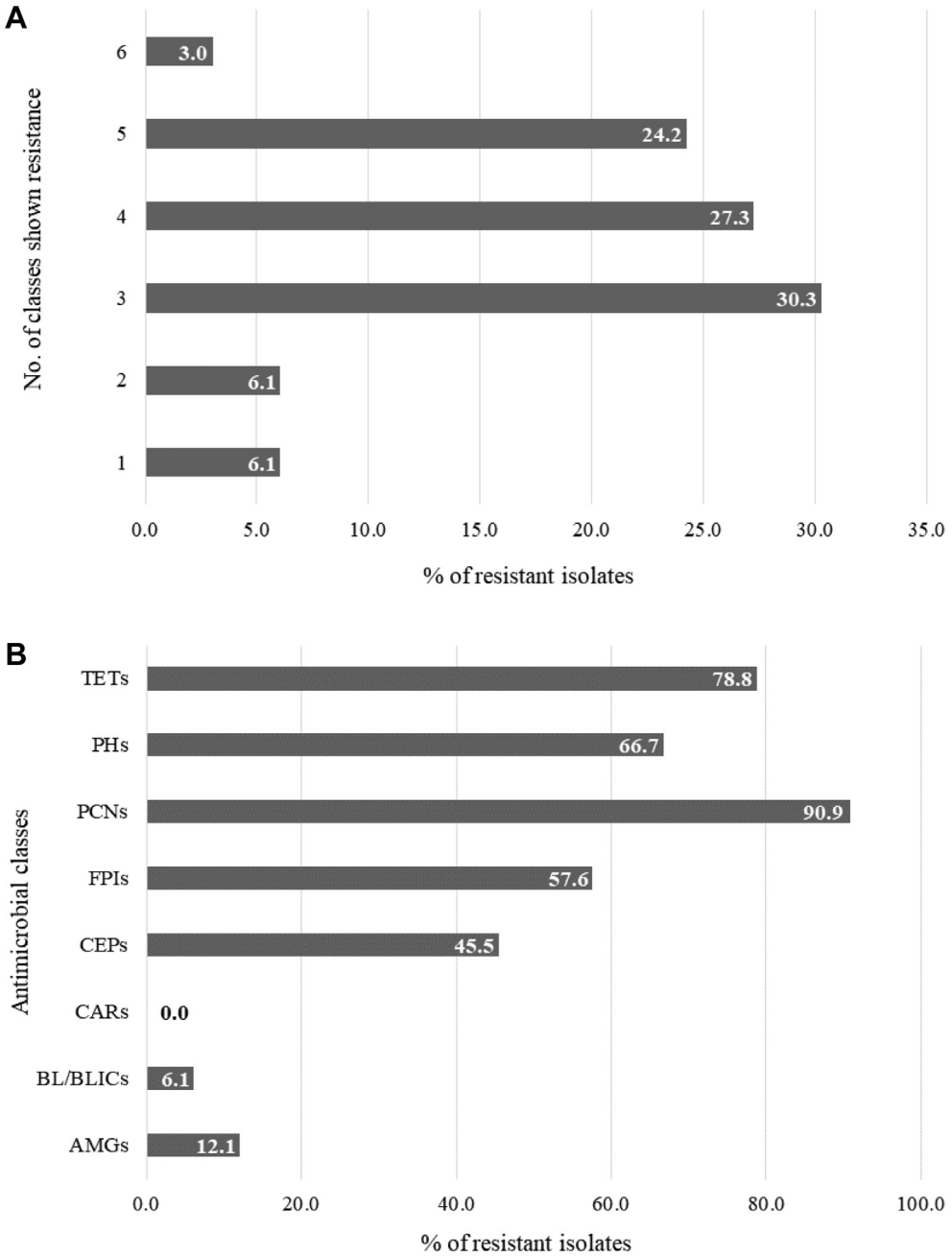

Figure 1. Antimicrobial resistance spectrum (A) and classes (B) in 33 fluoroquinolone-resistance avian pathogenic *E. coli* isolates. AMGs, aminoglycosides; BL/BLICs, β-lactam/β-lactamase inhibitor combinations; CARs, carbapenems; CEPs, cephems; FPIs, folate pathway inhibitors; PCNs, penicillins; PHs, 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 TEs (78.8%), phenicols (66.7%), folate pathway inhibitors (57.6%), cephems (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 $bla_{\text{CTX-1}}$ and $bla_{\text{CTX-15}}$, respectively. The non–extended-spectrum β-lactamase gene, $bla_{\text{TEM-1}}$, was found in 4 isolates. Of the 5 β-lactamase–producing

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

		Amino acid change		MIC (μg/mL)			
O Serotype	PMQR genes	*gyrA*	*parC*	CIP	ENR	Nor	No. of isolates included
O2	-[1]	S83 L/D87Y	S80 R	4	8	8	3
O3	-	S83 L/D87 N	S80I	8	16	16	1
O3	-	S83 L/D87 N	S80 R	4	16	16	1
O3	-	S83 L/D87 N	S80I	8	32	16	1
O9	*qnrA*	S83 L/D87 N	S80I	8	32	16	1
O9	*qnrA*	S83 L/D87 N	S80I	16	32	32	1
O45	-	S83 L/D87Y	S80I	16	128	64	1
O45	-	S83 L/D87 N	S80I/E84 G	64	128	>512	1
O78	*qnrA*	S83 L/D87 N	S80I	8	32	16	1
O78	-	S83 L/D87 N	S80I	16	32	64	1
O78	-	S83 L/D87 N	S80I	8	32	16	1
O78	-	S83 L/D87 N	S80I/E84 A	64	128	>512	1
O78	-	S83 L/D87 N	S80I/E84 G	64	128	>512	1
O86	-	S83 L/D87 N	S80I	128	128	256	1
O86	-	S83 L/D87 N	S80I	64	128	>512	1
O88	*qnrA*	S83 L/D87 N	S80I	8	32	16	1
O99	-	S83 L/D87 N	S80I	8	16	16	1
O104	-	S83 L/D87Y	S80 R	8	32	16	1
O111	-	S83 L/D87 N	S80I	8	32	16	1
O115	*qnrA*	S83I/D87 E	S80I	8	32	16	1
O128	-	S83 L/D87Y	S80I	16	64	64	1
O128	*qnrS*	S83 L/D87 N	S80I	16	32	128	1
O133	-	S83 L/D87 N	S80 R	8	16	16	1
O141	*qnrS*	S83 L/D87 N	S80I	16	32	16	1
O148	-	S83 L/D87 N	S80I	32	64	64	1
O166	-	S83 L/D87Y	S80I	8	32	16	1
Ogp8[2]	*qnrA*	S83 L/D87 N	S80I	8	32	16	1
Ogp8	*qnrS*	S83 L	S80I	16	32	16	1
ONT[3]	*qnrA*	S83 L/D87 N	S80I	64	128	256	1
ONT	*qnrA*	S83 L/D87 N	S80I	16	32	64	1
ONT	-	S83 L/D87 N	S80I	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.

[1]Not detected.

[2]O107 or O117.

[3]Untyped.

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	O Serotype
CC-23-10	*qnrA*	AM, AMC, CF, CL, CTX, CXM, CZ, CIP, SXT, TE	bla_{TEM-1}, bla_{CTX-1} *tetA*, *sul2*	I	FIB, HI1, I1, K/B	O88
CC-32-20	*qnrA*	AM, AMC, CAZ, CF, CL, CZ, FOX, CIP, TE	*tetA*	-[1]	B/O, FIB, K/B, N	O78
CC-32-48	*qnrA*	AM, CIP, SXT, TE	*tetA*, *sul2*	I (-)	FIB, Y	ONT[2]
CC-35-27	*qnrA*	AM, CF, CIP, TE	bla_{TEM-1}, *tetA*	I (*dfr*A17-*aad*A5)	FIB	O9
CC-35-46	*qnrA*	C, CIP, SXT, TE	*tetA*, *sul2*	I (*estX-psp-aad*A2)	B/O, FIB, K/B, N	Ogp8[3]
CC-35-47	*qnrA*	AM, C, CF, CL, CTX, CXM, CZ, FEP, CIP, SXT, TE	bla_{TEM-1}, bla_{CTX-15}, *tetA*, *sul1*	-	FIB	O9
CC-37-25	*qnrA*	AM, C, CIP	*catA1*	II (-)	FIB	O115
PS-35-14	*qnrA*	AM, C, CF, CL, CTX, CXM, CZ, GM, CIP, SXT, TE	bla_{CTX-15}, *tetA*	I (-)	FIB, I1	ONT
CC-9-91	*qnrS*	AM, CF, CL, CZ, CIP, TE	bla_{TEM-1}, *tetA*	I (*dfr*A12-*aad*A2)	FIA, FIC, Frep, N	O128
CC-34-53	*qnrS*	AM, C, GM, CIP, SXT	*aac(3)-II*, *sul1*, *catA1*	I (*dfr*A12-*aad*A2)	FIA, FIC, X	O141
CC-35-51	*qnrS*	AM, C, GM, CIP A, SXT	*sul1*	I (*dfrA27*)	K/B, HI2, W, Y	Ogp8

Underline indicate that was found in the transconjugant strains.

Abbreviations: AM, ampicillin; AMC, amoxicillin-clavulanate; C, chloramphenicol; CAZ, ceftazidime; CF, cephalothin; CIP, ciprofloxacin; CL, cephalexin; CTX, cefotaxime; CXM, cefuroxime; CZ, cefazolin; FEP, cefepime; FOX, cefoxitin; GM, gentamicin; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline.

[1]Not detected.

[2]Untyped.

[3]O107 or O117.

APEC isolates, 2 isolates had both the TEM and CTX-M genes. The other β-lactamase–encoding genes, SHV, OXA, and CTX-M group II, III, and IV, were not detected in any of the PMQR-positive isolates. Among 3 aminoglycoside-resistant isolates, *aac(3)-II* was only detected in one isolate. All 8 APEC isolates with resistance to TE carried the *tetA* gene, and the *tetB* and *tetC* genes were not present. Overall, 6 of the 7 trimethoprim-sulfamethoxazole–resistant isolates carried the *sul1* or *sul2* genes, and only 2 of the 8 C-resistant isolates carried the *catA1* gene.

Among 9 isolates with integrons, 8 isolates harbored class 1 integrons, and only 1 isolate harbored class 2 integrons. Class 1 integrons contained 4 types of gene cassettes, *dfr*A12-*aad*A2 (2 isolates), *dfr*A17-*aa*dA5 (1 isolate), *ext*X-*psp*-*aad*A2 (1 isolate), and *dfrA27* (1 isolate). Five isolates did not carry any of the gene cassettes. A total of 13 plasmid replicon types were identified in all 11 PMQR-positive FQ-resistant APEC isolates. The most common plasmid replicon was FIB (8 isolates, 72.7%), followed by K/B (4 isolates, 36.4%). Transferability was only identified in 8 isolates among 11 PMQR-positive FQ-resistant APEC isolates.

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 ug/mL), ENR (128 ug/mL), and NOR (>512 ug/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 μg/mL), ENR (128 μg/mL), and NOR (≥256 μg/mL), only one isolate carried the PMQR gene, *qnrA*. However, 11 PMQR-positive APEC isolates carried a variety of antimicrobial resistance genes such as $bla_{\text{CTX-1}}$, $bla_{\text{CTX-15}}$, $bla_{\text{TEM-1}}$, *aac(3)-II*, *tetA* *sul1*, *sul2*, and *catA1* 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 β-lactamase genes hydrolyze the characteristic β-lactam ring and confer resistance to most β-lactam antimicrobials, including cephalosporins (Paterson and Bonomo., 2005). The $bla_{\text{TEM-1}}$ gene code for narrow-spectrum β-lactamases 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 β-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 β-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, β-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 replicons. 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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REFERENCES

Ahmed, A. M., T. Shimamoto, and T. Shimamoto. 2013. Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. Int. J. Med. Microbiol. 303:475–483.

Animal and Plant Quarantine Agency (APQA). 2017. National Antimicrobial Resistance Monitoring program. APQA, Gimcheon, Republic of Korea.

Briñas, L., M. Zarazaga, Y. Sáenz, F. Ruiz-Larrea, and C. Torres. 2002. Beta-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. Antimicrob. Agents Chemother. 46:3156–3163.

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

Cavaco, L. M., H. Hasman, S. Xia, and F. M. Aarestrup. 2009. *qnrD*, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. Antimicrob. Agents Chemother. 53:603–608.

Chiu, C. H., T. L. Wu, L. H. Su, C. Chu, J. H. Chia, A. J. Kuo, M. S. Chien, and T. Y. Lin. 2002. The emergence in Taiwan of fluoroquinolone resistance in *Salmonella enterica* serotype choleraesuis. N. Engl. J. Med. 346:413–419.

CLSI. 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-Second Edition. CLSI Document M31-A2. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI. 2017. Performance Standards for Antimicrobial Susceptibility Testing. 27th Ed. CLSI Supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.

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

Dallenne, C., A. da Costa, D. Decré, C. Favier, and G. Arlet. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J. Antimicrob. Chemother. 65:490–495.

Dessie, H. K., D. H. Bae, and Y. J. Lee. 2013. Characterization of integrons and their cassettes in *Escherichia coli* and *Salmonella* isolates from poultry in Korea. Poult. Sci. 92:3036–3043.

Endtz, H. P., G. J. Ruijs, B. van Klingeren, W. H. Jansen, T. van der Reyden, and R. P. Mouton. 1991. Quinolone resistance in *campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J. Antimicrob. Chemother. 27:199–208.

Iguchi, A., S. Iyoda, K. Seto, T. Morita-Ishihara, F. Scheutz, and M. Ohnishi. 2015. *Escherichia coli* O-Genotyping PCR: a comprehensive and practical platform for molecular O serogrouping. J. Clin. Microbiol. 53:2427–2432.

Jahantigh, M., and R. E. Dizaji. 2015. Antimicrobial drug resistance pattern of Escherichia coli isolated from chickens farms with colibacillosis infection. Open J. Med. Microbiol. 5:159–162.

Jiang, Y., Z. Zhou, Y. Qian, Z. Wei, Y. Yu, S. Hu, and L. Li. 2008. Plasmid-mediated quinolone resistance determinants *qnr* and *aac(6')-Ib-cr* in extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in China. J. Antimicrob. Chemother. 61:1003–1006.

Johnson, J. R., M. A. Kuskowski, M. Menard, A. Gajewski, M. Xercavins, and J. Garau. 2006. Similarity between human and chicken *Escherichia coli* isolates in relation to ciprofloxacin resistance status. J. Infect Dis. 194:71–78.

Johnson, T. J., Y. Wannemuehler, C. Doetkott, S. J. Johnson, S. C. Rosenberger, and L. K. Nolan. 2008. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J. Clin. Microbiol. 46:3987–3996.

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.

Kim, M. S., H. M. Kwon, and H. W. Sung. 2009. Antibiotic resistance pattern of avian pathogenic *Escherichia coli* isolated from chickens. Korean J. Vet. Res. 49:195–200.

Kim, T. E., Y. W. Jeong, S. H. Cho, S. J. Kim, and H. J. Kwon. 2007. Chronological study of antibiotic resistances and their relevant genes in Korean avian pathogenic *Escherichia coli* isolates. J. Clin. Microbiol. 45:3309–3315.

Kim, Y. J., J. H. Park, and K. H. Seo. 2018. Comparison of the loads and antibiotic-resistance profiles of *Enterococcus* species from conventional and organic chicken carcasses in South Korea. Poult. Sci. 97:271–278.

Li, Y., L. Chen, X. Wu, and S. Huo. 2015. Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. Poult. Sci. 94:601–611.

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.

Magiorakos, A. P., A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, and S. Harbarth, et al. 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.

Matthijs, M. G., J. H. van Eck, W. J. Landman, and J. A. Stegeman. 2003. Ability of Massachusetts-type *infectious bronchitis virus* to increase colibacillosis susceptibility in commercial broilers: a comparison between vaccine and virulent field virus. Avian Pathol. 32:473–481.

Maynard, C., J. M. Fairbrother, S. Bekal, R. C. Levesque, R. Brousseau, L. Masson, and S. Larivie. 2003. Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* O149:K91 isolates obtained over a 23-year period from pigs. Antimicrob. Agents Chemother. 47:3214–3221.

Minarini, L. A., L. Poirel, V. Cattoir, A. L. Darini, and P. Nordmann. 2008. Plasmid-mediated quinolone resistance determinants among enterobacterial isolates from outpatients in Brazil. J. Antimicrob. Chemother. 62:474–478.

Ng, 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.

Oh, J. Y., M. S. Kang, J. M. Kim, B. K. An, E. A. Song, J. Y. Kim, E. G. Shin, M. J. Kim, J. H. Kwon, and Y. K. Kwon. 2011. Characterization of *Escherichia coli* isolates from laying hens with colibacillosis on 2 commercial egg-producing farms in Korea. Poult. Sci. 90:1948–1954.

Oh, J. Y., Y. K. Kwon, M. D. Tamang, H. K. Jang, O. M. Jeong, H. S. Lee, and M. S. Kang. 2016. Plasmid-mediated quinolone resistance in *Escherichia coli* isolates from wild birds and chickens in South Korea. Microb. Drug Resist 22:69–79.

Paterson, D. L., and R. A. Bonomo. 2005. Extended-spectrum beta-lactamases: a clinical update. Clin. Microbiol. Rev. 18:657–686.

Pitout, J. D., A. Hossain, and N. D. Hanson. 2004. Phenotypic and molecular detection of CTX-M-beta-lactamases produced by *Escherichia coli* and *Klebsiella spp.* J. Clin. Microbiol. 42:5715–5721.

Poirel, L., J. Y. Madec, A. Lupo, A. K. Schink, N. Kieffer, P. Nordmann, and S. Schwarz. 2018. Antimicrobial resistance in *Escherichia coli*. J. Environ. Health 70:40–45.

Pons, M. J., S. Mosquito, C. Gomesa, L. J. Del Valle, T. J. Ochoa, and J. Ruiz. 2014. Analysis of quinolone-resistance in commensal and diarrheagenic *Escherichia coli* isolates from infants in Lima, Peru. Trans. R. Soc. Trop. Med. Hyg. 108:22–28.

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 Origins. Antimicrob. Agents Chemother. 48:3996–4001.

Sandvang, D., and F. M. Aarestrup. 2000. Characterization of aminoglycoside resistance genes and class 1 integrons in porcine and bovine gentamicin-resistant *Escherichia coli*. Microb. Drug Resist 6:19–27.

Sandvang, D., F. M. Aarestrup, and L. B. Jensen. 1998. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. FEMS Microbiol. Lett. 160:37–41.

Sengeløv, G., Y. Agersø, B. Halling-Sørensen, S. B. Baloda, J. S. Andersen, and L. B. Jensen. 2003. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environ. Int. 28:587–595.

Seo, K. W., and Y. J. 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. Detection of plasmid-mediated quinolone resistance genes in β-lactamase-producing *Escherichia coli* isolates from layer hens. Poult. Sci. 98:1480–1487.

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. 56:2705–2712.

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.

Van, T. T. H., J. Chin, T. Chapman, L. T. Tran, and P. J. Coloe. 2008. Safety of raw meat and shellfish in Vietnam: an analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. Int. J. Food Microbiol. 124:217–223.

Varela, A. R., G. N. Macedo, O. C. Nunes, and C. M. Manaia. 2015. Genetic characterization of fluoroquinolone resistant *Escherichia coli* from urban streams and municipal and hospital effluents. FEMS Microbiol. Ecol. 91:fiv015.

Varga, C., M. L. Brash, D. Slavic, P. Boerlin, R. Ouckama, A. Weis, M. Petrik, C. Philippe, M. Barham, and M. T. Guerin. 2018. Evaluating virulence-associated genes and antimicrobial resistance of avian pathogenic *Escherichia coli* isolates from broiler and broiler breeder chickens in Ontario, Canada. Avian Dis. 62:291–299.

Wang, M., J. H. Tran, G. A. Jacoby, Y. Zhang, F. Wang, and D. C. Hooper. 2003. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob. Agents Chemother. 47:2242–2248.

Wu, S., A. Dalsgaard, A. M. Hammerum, L. J. Porsbo, and L. B. Jensen. 2010. Prevalence and characterization of plasmids carrying sulfonamide resistance genes among *Escherichia coli* from pigs, pig carcasses and human. Acta Vet. Scand. 52:47–53.

Xue, G., J. Li, Y. Feng, W. Xu, S. Li, C. Yan, H. Zhao, and H. Sun. 2017. High prevalence of plasmid-mediated quinolone resistance determinants in *Escherichia coli* and *Klebsiella pneumoniae* isolates from pediatric patients in China. Microb. Drug Resist 23:107–114.

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, 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. 6:964–972.

Yeh, J. C., D. Y. Lo, S. K. Chang, C. C. Chou, and H. C. Kuo. 2017. Prevalence of plasmid-mediated quinolone resistance in *Escherichia coli* isolated from diseased animals in Taiwan. J. Vet. Med. Sci. 79:730–735.