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Short communication

Characterization of plasmid mediated quinolone resistance determinants in ciprofloxacin resistant-*Escherichia coli* from chicken meat produced by integrated broiler operations in Korea

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ABSTRACT

The purpose of this study was to determine the genetic characterization of ciprofloxacin resistant- *Escherichia coli* recovered from 7 different integrated broiler operations in Korea. Among the 157 *E. coli* isolated from chicken meat produced by integrated broiler operations, 75 (47.8%) were observed to be ciprofloxacin resistant-*E. coli*. However, the prevalence varied from 25.0 to 75.0%, in chicken meat, indicating variation in ciprofloxacin resistant *E. coli* occurrence among the operations. Among the 75 ciprofloxacin resistant-*E. coli* isolates, 10 showed plasmid-mediated quinolone resistance (PMQR) genes, *aac(6′)-Ib-cr*, *qnrS1* and *qnrB4*. Among the 10 PMQR-positive *E. coli*, a double amino-acid exchange in both *gyr*A and *par*C with ciprofloxacin minimum inhibitory concentrations of ≥16 μg/mL was noted in 8 isolates, and 4 transconjugants (40.0%) expressed similar antimicrobial resistance patterns and revealed the presence of PMQR genes and β-lactamase genes. Our findings suggest that *E. coli* with resistance to ciprofloxacin can now be found in association with integrated broiler operations, thus highlighting the need for monitoring and prevention programs in integrated operations.

1. Introduction

Escherichia coli (*E. coli*) is a common pathogen that affects both animals and humans, and different quinolones and fluoroquinolones have been approved for the treatment of several animal diseases around the world (Markland et al., 2015; Wang et al., 2015). However, in many countries, the prevalence of quinolone-resistant *E. coli* isolates has increased in humans, food animals such as pigs, cattle, and poultry, and particularly in retail chicken meat (Ghodousi et al., 2015; Zhao et al., 2012). Quinolone resistance is mainly attributed to chromosomal mutations that alter the drug target enzymes DNA gyrase and DNA topoisomerase IV, or activate the efflux systems (Kim et al., 2013). However, plasmid-mediated quinolone resistance (PMQR) genes have been recently identified in various Enterobacteriaceae, and their prevalence is increasing worldwide (Poirel et al., 2012).

In Korea, several large integrated broiler operations supply about 80% of marketed broiler chickens (KAPE, 2015). The livestock on the integrated farms, which includes chicken, is reared intensively, with antimicrobial agents used as growth promoters and for prophylactic as well as therapeutic treatments. In previous studies, the prevalence and antimicrobial resistance patterns of food-poisoning pathogens varied among different integrated broiler operations (Kim et al., 2012; Kim et al., 2018). However, the prevalence and characterization of ciprofloxacin resistant-*E. coli* isolated from integrated broiler operations has not yet been reported in Korea. The primary objective of this study was to genetically characterize ciprofloxacin resistant-*E. coli* recovered from 7 different integrated broiler operations in Korea.

2. Materials and methods

For *E. coli* isolation, a total of 200 fresh packed chicken meats (carcasses) were collected during 2016. These meats were produced by 50 broiler farms and divided into 7 different integrated broiler operations [A (n = 56), B (n = 44), C (n = 32), D (n = 32), E (n = 12), F (n = 12) and G (n = 12)] which supplied about 80% of the broiler chickens in Korea. Four meats from each farm origin were sampled for this study. Microbiological analyses were performed according to the Processing and Ingredients Specification of Livestock Products published by Ministry of Food and Drug Safety (KFDS, 2014). Final confirmation of *E. coli* was carried by PCR as previously described (Candrian et al., 1991). If isolates from the same farm showed the same antimicrobial susceptibility patterns, only one isolate was randomly

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chosen and included in this study. Therefore, a total of 157 *E. coli* isolates originated from 7 different integrated broiler operations were tested in this study: A (n = 33), B (n = 41), C (n = 24), D (n = 18), E (n = 9), F (n = 16) and G (n = 16) broiler operations. All *E. coli* isolates were investigated for their antimicrobial resistance with the disc diffusion test using amoxicillin–clavulanate (20/10 μg), ampicillin (10 μg), cefadroxil (30 μg), cefalexin (30 μg), cefazolin (30 μg), cefepime (30 μg), cefotaxime (30 μg), cefovecin (30 μg), cefoxitin (30 μg), ceftazidime (30 μg), ceftiofur (30 μg), cefuroxime (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), imipenem (10 μg), nalidixic acid (30 μg), tetracycline (30 μg), and trimethoprim–sulfamethoxazole (1.25/23.75 μg) discs (BD Biosciences, Sparks, MD, USA) according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2013). The minimum inhibitory concentrations (MICs) to ciprofloxacin (CIP), nalidixic acid and enrofloxacin (ENR) at concentrations ranging from 0.06 to 512 μg/mL were determined by standard agar dilution methods with Mueller-Hinton agar (BD) according to the recommendations of the CLSI (CLSI, 2013). Multi-drug resistance (MDR) was defined as acquired non-susceptibility to at least 1 agent in 3 or more antimicrobial categories.

PCR amplification of the PMQR markers (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6′)-Ib-cr* and *qepA*) and β-lactamase genes ($bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, and bla_{OXA}) were carried out as previously described (Briñas et al., 2002; Pitout et al., 2004; Yu et al., 2015). Conjugation experiments to determine the transferable capacity of plasmids carrying PMQR determinants and β-lactamase genes, and the frequency of mutations in the quinolone-resistance determining region (QRDR) were performed as specified by Rodríguez-Martínez et al. (2006) and Vasilaki et al. (2008), respectively.

3. Results

Among the 157 *E. coli* isolates from chicken meat produced by 7 integrated broiler operations, 75 (47.8%) were observed to be ciprofloxacin resistant-*E. coli*. However, ciprofloxacin resistant-*E. coli* prevalence varied from 25.0 to 75.0%, revealing that variation in *E. coli* levels occurs among the different operations (Fig. 1). Especially, ciprofloxacin resistant-*E. coli* from the operation G had the highest prevalence (75.0%, 12 of 16 isolates), whereas operation C had the lowest prevalence (25.0%, 6 of 24 isolates). The MDR-*E. coli* levels also showed varied prevalence from 75.0 to 100.0%.

Among the 75 ciprofloxacin resistant-*E. coli* isolates, 10 (13.3%) were PMQR-positive *E. coli* (Table 1). Two *qnr* genes, *qnrS1* and *qnrB4*, were identified in 3 and 6 *E. coli* isolates, respectively, while one *aac (6′)-Ib-cr* gene was detected. Among the 10 PMQR-positive *E. coli* isolates, 3 β-lactamase genes, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-14}}$, and $bla_{\text{TEM-1}}$, were identified in 1, 2 and 5 *E. coli*, respectively.

In the 10 PMQR-positive *E. coli*, only 4 transconjugants (40.0%) showed a transferability of PMQR genes, β-lactamase genes, and similar antimicrobial resistance. Eight PMQR-positive isolates showed double amino acid exchange at both *gyr*A and *par*C with CIP, and ENR MICs of ≥16 μg/mL and ≥32 μg/mL, respectively, while 1 PMQR-positive isolate revealed single amino acid exchange at *gyr*A with CIP, and ENR MICs of ≥8 μg/mL and ≥16 μg/mL, respectively. Only one PMQR-positive isolate had wild type in both *gyr*A and *par*C with CIP and ENR MICs ≥ 4 μg/mL in both (Table 1).

4. Discussion

Ciprofloxacin resistance develops when bacteria change in response to the use of these medicines (Jacoby, 2005). If fluoroquinolones including CIP and ENR have been continuously used in operations, the prevalence of ciprofloxacin resistant-*E. coli* may be due to differences in the usage of antibiotics at each operation. Additionally, widespread use of antibiotics in the poultry industry to promote growth and prevent microbial infections has led to the emergence of MDR *E. coli* strains (Gelband et al., 2015). In this study, the prevalence of MDR *E. coli* isolated from different integrated broiler operations varied and the patterns of these isolates differed depending on the operations of chicken meat. This study indicates that mass medication has been continuously used in each operation in Korea, and therefore, regulation on the use of antibiotics in livestock, as noted in other countries, is required in Korea (Schmidt, 2012).

The prevalence of PMQR determinants among *E. coli* isolates from

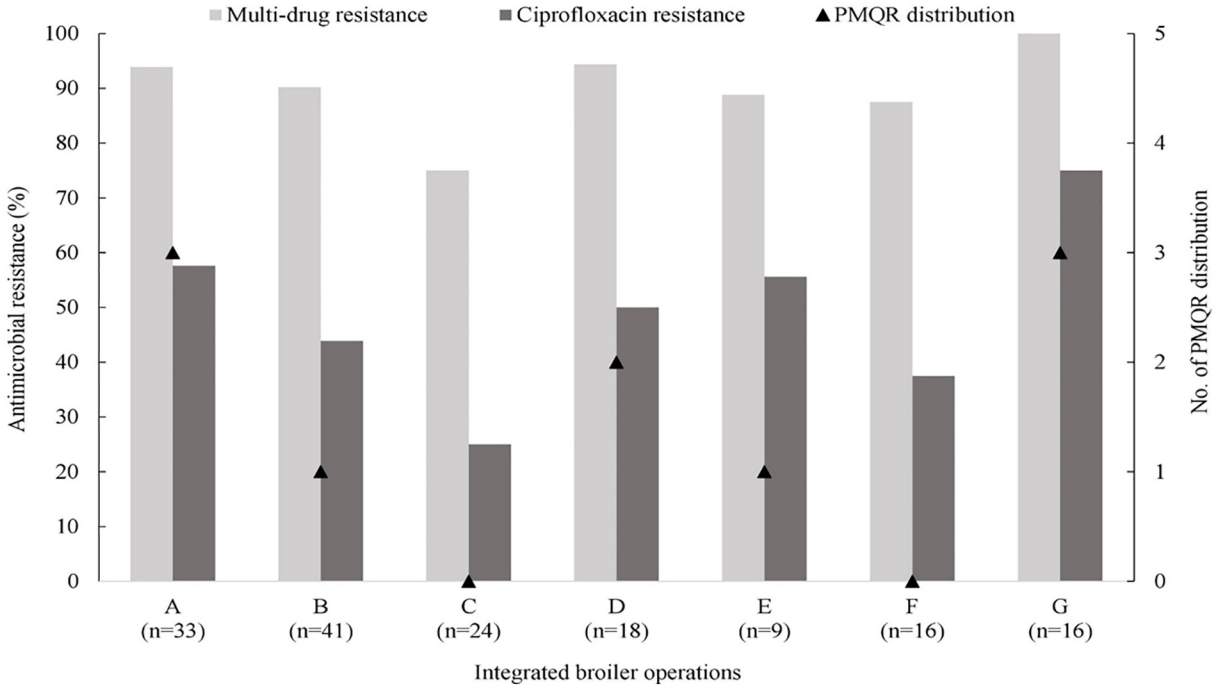

Fig. 1. Prevalence of multi-drug and ciprofloxacin resistance (hatched bars) and distribution of the PMQR genes (▲) among 157 isolates from 7 integrated broiler operations. PMQR: plasmid-mediated quinolone resistance.

Table 1
Characteristics of the 10 PMQR-positive *E. coli* isolated from chicken meat produced by integrated broiler operations.

Integrated broiler operations	Isolate	PMQR[a] genes	MIC (mg/L)[b]			QRDR mutations[c]		β-lactamase genes	Antimicrobial resistance pattern[b]
			NA	ENR	CIP	*gyrA*	*parC*		
A	CE-10[d]	*qnrS1*	≥512	4	4	WT	WT	CTX-M-14	AM, CZ, CL, CFR, CF, CTX, CVN, EFT, C, G, SXT, TE
	T-CE-10[e]	*qnrS1*	4	0.06	0.06	NT	NT	CTX-M-14	AM, CZ, CF, CTX, C, G, SXT, TE
	CE-51	*qnrS1*	≥512	32	16	S83L, D87N	S80I	TEM-1	AM, AMC, CZ, CL, CFR, CF, FOX, CVN, C, G, IPM, SXT, TE
	CE-54[d]	*qnrS1*	≥512	256	128	S83L, D87N	S80I	TEM-1	AM, AMC, CZ, CL, CFR, CF, FOX, CVN, C, SXT, TE
	T-CE-54[e]	*qnrS1*	8	0.125	0.5	NT	NT	TEM-1	AM, AMC, CZ, CFR, C, SXT
B	CE-20	*qnrB4*	≥512	512	≥512	S83L, D87N	S80I, E84G	CTX-M-1, TEM-1	AM, AMC, CZ, CL, CFR, CF, FOX, CXM, CAZ, CTX, CVN, EFT, FEP, C, IPM, SXT, TE
D	CE-12	*qnrB4*	≥512	32	16	S83L, D87N	S80I	–	AM, CZ, CL, CFR, CF, C, G, SXT, TE
	CE-63	*aac(6′)-Ib-cr*	≥512	128	32	S83L, D87N	S80I, E84A	–	CF, C, SXT, TE
E	CE-24	*qnrB4*	≥512	256	128	S83L, D87N	S80I, E84G	–	CL, CF, C, IPM
G	CE-15[d]	*qnrB4*	≥512	32	16	S83L, D87N	S80I	TEM-1	CL, CFR, CF, EFT, SXT, TE
	T-CE-15[e]	*qnrS1*	2	0.125	0.06	NT	NT	TEM-1	CF, SXT, TE
	CE-65[d]	*qnrB4*	≥512	64	32	S83L, D87N	S80R	TEM-1	AM, AMC, CZ, CF, CVN, G, TE
	T-CE-65[e]	*qnrS1*	8	0.5	0.25	NT	NT	TEM-1	AMC, CZ, CF, G, TE
	CE-69	*qnrB4*	≥512	16	8	S83L	WT	CTX-M-14	AM, CZ, CL, CFR, CF, CXM, CTX, CVN, EFT

[a] PMQR, plasmid-mediated quinolone resistance.
[b] NA, nalidixic acid; ENR, enrofloxacin; CIP, ciprofloxacin; AM, ampicillin; AMC, amoxicillin-clavulanic acid; CZ, cefazolin; CL, cefalexin; CFR, cefadroxil; CF, cephalothin; FOX, Cefoxitin; CXM, cefuroxime; CAZ, ceftazidime; CTX, cefotaxime; CVN, cefovecin; EFT, ceftiofur; FEP, cefepime; C, chloramphenicol; G, gentamicin; IPM, imipenem; SXT, sulfamethoxazole/trimethoprim; TE, tetracycline.
[c] QRDR, quinolone-resistance determining region; WT, wild type; NT, not tested.
[d] Donor.
[e] Transconjugant.

humans, animals and the environment has been described in many studies (Chen et al., 2012; Han et al., 2010). In this study, although PMQR genes were not detected in operation C and F, 10 isolates identified as PMQR-positive *E. coli* in 5 integrated broiler operations. Generally, PMQR genes confer low-level resistance to quinolones, but they can play a significant role in the generation of resistant mutations and the spread of antimicrobial resistance (Yang et al., 2008). In this study, the QRDRs of *gyrA* and *parC* were sequenced in PMQR-positive isolates to establish the association between chromosomal mutation and the presence of these PMQR determinants. Among 10 PMQR-positive *E. coli*, 8 isolates had a double amino-acid exchange in both *gyrA* and *parC*, with CIP MICs of ≥16 μg/mL. The extensive use of fluoroquinolones has led to the emergence of fluoroquinolone-resistant *E. coli*, with a particularly higher level of resistance to fluoroquinolones among double mutants (Briales et al., 2012).

In this study, transconjugants expressed similar antimicrobial resistance patterns and revealed the presence of PMQR genes and β-lactamase genes. This is also consistent with a previous study which suggested that transconjugants have the same genes and similar antibiotic resistance patterns as the donor strains (Kim et al., 2013; Yu et al., 2015). It indicates that the dissemination of PMQR genes and β-lactamase genes were possibly due to the transmission of plasmids through horizontal transfer and chicken meat can contribute to the transmission of these genes to humans (Thorsteinsdottir et al., 2010).

To our knowledge, this is the first study to evaluate the characterization of ciprofloxacin resistant-*E. coli* isolated from different integrated chicken operations in Korea. Our findings suggest that *E. coli* with resistance to ciprofloxacin are present in integrated broiler operations, and that the surveillance-and-intervention strategy must include investigation and identification of management factors that affect the presence of these pathogens in integrated operations.

Declaration of Competing Interest

All authors declare no competing interest.

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