creative commons
COMMONS DEED

저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.

변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

dCollection

수의학석사학위논문

Fluoroquinolone antimicrobial resistance of *Escherichia coli* isolated from swine feces in Korea

국내 돼지 분변 유래 대장균 분리주의 플루오로퀴놀론 항생제 내성 연구

2017년 2월

서울대학교 대학원
수의학과 수의미생물학 전공
허 윤 성

Fluoroquinolone antimicrobial resistance of *Escherichia coli* isolated from swine feces in Korea

By

Yoon Sung Hu

February, 2017

Department of Veterinary Medicine

(Major: Veterinary Microbiology)

The Graduate School

Seoul National University

Fluoroquinolone antimicrobial resistance of *Escherichia coli* isolated from swine feces in Korea

By

Yoon Sung Hu

Adviser: Prof. Yong Ho Park

A dissertation submitted to the faculty of the
Graduate School of Seoul National University
in partial fulfillment of the requirement for
the degree of Master in Veterinary Microbiology

February 2017

Department of Veterinary Medicine
(Major: Veterinary Microbiology)
The Graduate School
Seoul National University

수의학석사학위논문

Fluoroquinolone antimicrobial resistance of *Escherichia coli* isolated from swine feces in Korea

국내 돼지 분변 유래 대장균 분리주의 플루오로퀴놀론 항생제 내성 연구

지도교수: 박 용 호

이 논문을 수의학석사 학위논문으로 제출함
2016 년 10 월

서울대학교 대학원 수의학과
수의미생물학 전공
허 윤 성

허윤성의 수의학 석사 학위논문을 인준함
2016 년 12 월

위 원 장: 조 성 범 (인)
부위원장: 박 용 호 (인)
위 원: 박 건 택 (인)

Fluoroquinolone antimicrobial resistance of *Escherichia coli* isolated from swine feces in Korea

Yoon Sung Hu
(Supervised by Prof. Yong Ho Park)
Department of Veterinary Medicine, The Graduate School of
Seoul National University

Abstract

In this study, the fluoroquinolone (FQ) resistance rate and mechanisms in FQ-resistant *Escherichia coli* isolated from swine fecal samples have been investigated. A total of 171 *E. coli* isolates were collected from 237 swine fecal samples (72.2%). Of these, 59 isolates (34.5%) were confirmed as FQ-resistant *E. coli* by the disk diffusion method. Of the FQ-resistant isolates, three major FQ-resistance mechanisms were investigated: i) amino acid substitutions in quinolone resistance-determining regions (QRDRs), ii) acquisition of plasmid-mediated quinolone-resistance genes (PMQRs; *qnrA*, *qnrB*, *qnrS*, *qepA*, and *aac(6')-Ib-cr*), and iii) increased efflux pump activity. All 59 ciprofloxacin (CIP)-resistant isolates had *gyrA* mutations (100%); of these 59 isolates, 58 had mutations in *parC* (98.3%), 22 had mutations in *parE* (37.3%), and none had mutations in *gyrB*. The predominant mutation was Ser83Leu in *gyrA*, followed by Ser80Ile in *parC*, and Ser458Ala in *parE*. Nine isolates harbored PMQR genes, including *qnrS* (n = 7, 11.9%), *qepA* (n = 1, 1.7%), and *aac(6')-Ib-cr* (n =

1, 1.7%). Efflux pump activity was found in 56 isolates (94.9%). FQ-resistant *E. coli* had high minimum inhibitory concentrations against CIP, and most isolates were multidrug-resistant. Compared with previous studies in Korea, the prevalence of FQ resistance and PMQR genes had increased significantly in swine. Although the use of FQ as a feed additives is prohibited in Korea, use for self-treatment and therapeutic purposes has been increasing, which may be responsible for the higher FQ-resistance rate observed in this study. Therefore, prudent use of FQ in animal farms is needed to reduce the evolution of FQ-resistant bacteria in Korea.

Keywords: quinolone, fluoroquinolone, antimicrobial resistance, *Escherichia coli*, pig

Student ID: 2015-21825

CONTENTS

LIST OF TABLES

INTRODUCTION

Fluoroquinolones (FQs) are some of the most frequently used antimicrobials worldwide for the treatment of both gram-negative and gram-positive bacterial infections *(2)*. The World Health Organization (WHO) has classified quinolones and FQs as "critically important antimicrobials" because of their broad-spectrum effects and clinical importance in both human and animal medicine *(19)*. Because the importance of FQs in humans and animals is increasing, FQ-resistant bacteria are a major concern in the treatment of infectious diseases.

Antimicrobials have an important role in the food-producing animal industry because of the use of feed additives to promote growth and prevent disease and for therapeutic purposes *(15)*. For these reasons, antimicrobials have been used widely in the food production industry. On an average, the use of antimicrobials is higher in the animal industry than in human medicine *(34)*. The swine industry is the most important livestock industry in South Korea, and the consumption of antimicrobials in the swine industry has been estimated to represent approximately 60% of all antimicrobial consumption in the animal industry in Korean *(18)*. Consequently, the antimicrobial-resistant rate in swine is relatively higher than that in other food-producing animals in Korea *(17, 21)*.

In an effort to reduce antimicrobial resistance in food-producing animals, many antimicrobial agents, including FQs, have been prohibited for use in feed

additives since 2009 in Korea *(21)*. Consequently, FQ-resistant bacteria were expected to decrease in the animal industry in Korea. Accordingly, in this study, the prevalence of FQ-resistant *Escherichia coli* isolated from swine feces have been investigated. In addition, three major FQ-resistance mechanisms in FQ-resistant *E. coli* have been characterized as follow: (i) target-enzyme modification (mutations in quinolone resistance-determining regions [QRDRs]), (ii) plasmid-mediated quinolone-resistance gene (PMQR) activity, and (iii) extrusion of drug agents by efflux pump activity *(38)*.

MATERIALS AND METHODS

1. Isolation and identification of FQ-resistant *E. coli*

A total of 237 swine fecal samples were collected from 24 swine farms in 6 provinces in Korea from March to June 2015 (Table 1). Of 237 samples, 53 (22.4%) were from swine with diarrhea. The other 184 samples (77.6%) were collected from healthy swine. All fecal samples were streaked on Columbia agar containing 5% sheep blood (BioMerieux SA, France) *(5)*, and candidate *E. coli* colonies were inoculated on MacConkey agar plates (BD, Sparks, MD, USA) and Triple Sugar Iron agar (BD) *(24)*. These inoculated plates were incubated at 37℃ for 24 h. Polymerase chain reaction (PCR) targeting the 16S ribosomal RNA region was performed to identify *E. coli* as described previously *(28)*. Only one *E. coli* isolate per sample was selected for further analysis.

For all isolated *E. coli*, antimicrobial susceptibility tests were performed against CIP to detect FQ-resistant *E. coli* using the standard disk diffusion test. FQ-resistant *E. coli* were identified according to the Clinical and Laboratory Standards Institute (CLSI) guideline *(33)*.

2. Minimum inhibitory concentrations (MICs) of nalidixic acid (NA) and ciprofloxacin (CIP)

Minimum inhibitory concentrations (MICs) of NA and CIP for FQ-resistant *E. coli* were determined by the broth microdilution method according to the CLSI guideline *(33)*. The concentrations of these two antimicrobial agents were 0.008–1024 μg/mL. *E. coli* ATCC 25922 was used as a reference strain.

3. Antibiogram of FQ-resistant *E. coli* against other antimicrobials

Antimicrobial resistance profiles of FQ-resistant *E. coli* were analyzed using the following antimicrobial disks (BD): amoxicillin (AM, 10 μg), amoxicillin-clavulanic acid (AMC, 20/10 μg), cefotaxime (CTX, 30 μg), ceftriaxone (CRO, 30 μg), cefotetan (CTT, 30 μg), ceftazidime (CAZ, 30 μg), imipenem (IMP, 10 μg), aztreonam (ATM, 30 μg), gentamicin (GM, 10 μg), tetracycline (TE, 30 μg), CIP (5 μg), NA (30 μg), trimethoprim-sulfamethoxazole (SXT, 1.25/23/75 μg), and chloramphenicol (C, 30 μg). The tests were performed according to the CLSI guideline *(33)*. *E. coli* ATCC 25922 was included in the test as a control strain. *E. coli* isolates showing resistance to more than three different classes of antimicrobials were defined as multidrug resistant (MDR) *E. coli* according to the previous terminology *(13)*. FQ-resistant *E. coli* isolates were further tested for production of extended-spectrum beta-lactamase (ESBL) by the combination

disk method, which compares the inhibition zone diameters of CTX and CTX/CL and of CAZ and CAZ/CL *(3)*.

4. Identification of mutations in QRDRs and detection of PMQRs

DNA samples for polymerase chain reaction (PCR) amplification were prepared from all FQ-resistant *E. coli* using a standard heat lysis protocol *(37)*. All PCR primers used in this study are listed in Table 2. For detection of gene mutations, each QRDR (*gyrA*, *gyrB*, *parC*, and *parE*) was first amplified by PCR, and the PCR fragments were custom-sequenced by Biofact Co. (Seoul, Korea). Mutations in QRDRs were identified by comparing the sequencing data with those of the *E. coli* K-12 strain (GenBank accession no. U00096).

PMQR genes (*qnrA*, *qnrB*, *qnrS*, *qepA*, and *aac(6')-Ib-cr*) were detected by PCR amplification and sequencing analysis, as described in previous studies *(7, 20, 35, 36)*.

5. Organic solvent tolerance (OST) test

OST tests were carried out to identify phenotypic efflux pump activity *(32)*. A single colony was inoculated in 2 mL Luria-Bertani (LB) broth for 5.5 h to reach the logarithmic phase. Cultures in the logarithmic phase were diluted to an optical density 0.2 (530 nm). Next, 100 μL of diluted culture was spread on LB

agar, and 10 mL of an organic solvent (hexane-cyclohexane [3:1]) was overlaid. The plates were sealed with parafilm to prevent evaporation and incubated at 30℃ overnight. The following day, the colony numbers were counted, and the results were analyzed as follows: strong efflux pump activity (++), ≥ 100 colonies; weak efflux pump activity (+), 1–99 colonies; no efflux pump activity (-), no growth. The ATCC 25922 strain was included as a reference strain.

6. Statistical analysis

All statistical analyses were performed using SPSS software version. 23 (SPSS Inc., USA). The numbers of ESBL and MDR isolates between low- and high CIP MIC groups were compared by Chi-squired test. P value less than 0.05 was considered significant. The correlations between OST and CIP MICs were analyzed by Pearson's correlation coefficient tests. As the R-value reached 1.0, the two data were assumed to be strongly correlated *(16)*.

RESULTS

1. Isolation of FQ-resistant *E. coli* and antimicrobial susceptibility tests

E. coli was isolated from 171 (72.2%) of 237 swine fecal samples. Among the 171 *E. coli* isolates, 52 (30.4%) were from diseased swine, and the other 119 (69.6%) were from healthy swine. Of the 171 isolates, 59 (34.5%) were confirmed as FQ-resistant *E. coli*, among which 21 (21/52; 40.4%) were from diseased swine and 38 (38/119; 31.9%) were from healthy swine. Fifty-four isolates (91.5%) were classified as MDR (Table 4). The rates of resistance to seven different classes of drugs are described in Table 4. Eleven isolates (18.6%) were confirmed as ESBL-producing *E. coli*. FQ-resistant isolates had MIC values against CIP ranging from 4 to 256 μg/mL, and all of these isolates were phenotypically resistant to NA (256 μg/mL and > 1024 μg/mL; Table 5).

2. Correlation of CIP MIC and frequencies of MDR and ESBL *E. coli*

The FQ resistant *E. coli* isolated in this study were divided into two groups, based on CIP MIC of each isolate, to analyze the relationship between CIP MIC and frequency of MDR and ESBL *E. coli* as following, low CIP MIC group (MIC was less than or equal to16 μg/mL) and high CIP MIC group (MIC was higher than 16 μg/mL). The frequencies of MDR and ESBL isolates were compared between the two groups. The frequencies of MDR (82.1 %) and ESBL

(100 %) in high MIC group were significantly higher than those (3.6 and 32.3 %, respectively) in low MIC group ($P < 0.05$ in both cases, by chi-squired test, Table 6).

3. Presence of amino acid substitutions in QRDRs in FQ-resistant *E. coli*

DNA sequences were analyzed to target the QRDRs for DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*). All 59 isolates had at least one site of QRDR amino acid substitutions in *gyrA*, *parC*, and *parE*. There were no *gyrB* gene mutations in any of the isolates in this study. One isolate (1.7%) had a single mutation in *gyrA*, 36 isolates (61.0%) had mutations in both *gyrA* and *parE*, and 22 isolates (37.3%) had mutations in *gyrA*, *parC*, and *parE* (Table 5). The *gyrA* amino acid substitutions were as follows: Ser83Leu (100%), Asp87Asn (69.5%), Asp87Gly (11.9%), Asp87Tyr (11.9%), and Asp87His (3.4%), and the *parC* mutations were as follows: Ala56Thr (8.6%), Ser80Ile (96.6%), Ser80Arg (1.7%), Glu84Gly (12.1%), Glu84Ala (8.6%), and Glu84Lys (1.7%). In *parE*, Ile355Thr (22.7%), Leu416Phe (4.5%), Ser458Ala (54.5%), Glu460Lys (13.6%), and Ile464Phe (9.1%) were observed.

4. Detection of PMQR genes in FQ-resistant *E. coli*

Nine of 59 FQ-resistant isolates (15.3%) harbored PMQR genes (*qnrS*, *qepA*, and *aac(6')-Ib-cr*), whereas none of these isolates had *qnrA* or *qnrB* genes. Each of the nine isolates harbored only one of the PMQR genes screened. The predominant PMQR gene detected in this study was *qnrS* (seven isolates), whereas *qepA* and *aac(6')-Ib-cr* were found in only one isolate each.

5. Phenotypic efflux pump activity

Phenotypic efflux pump activity was measured by OST tests *(32)*. Most of the FQ-resistant *E. coli* (56 isolates, 94.9%) showed efflux pump activity against organic solvent. Among the isolates with positive efflux pump activity, 40 isolates (67.8%) were found to have strong efflux pump activity, and 16 isolates (27.1%) had moderate activity. Notably, the CIP MIC was not correlated with phenotypic efflux pump activity using Pearson's correlation coefficient (R-value: 0.044, data not shown).

DISCUSSION

FQ has been prohibited as a feed additive since 2009 in an effort to reduce antimicrobial resistance in food-producing animals in Korea *(21)*. However, the current study indicated that FQ resistance in *E. coli* (34.5%) isolated from pigs from Korea was higher than that from other countries *(23, 29)* and has significantly increased within the last decade *(17)*. This is largely due to the massive use of FQ in livestock for therapeutic and self-treatment purposes, instead of feed additives; thus, the total amounts of FQ did not change substantially before and after the ban of FQ as a feed additive *(21)*. Therefore, additional regulations reducing the use of FQ in food-producing animals may be needed to reduce FQ resistance in Korea.

To date, three mechanisms through which bacteria acquire FQ resistance have been reported. One of the most important resistance mechanisms is drug target modifications in DNA gyrase and topoisomerase IV. Gyrase is encoded by *gyrA* and *gyrB*, and topoisomerase IV is encoded by *parC* and *parE (12)*. Because DNA gyrase is the primary target of FQ in gram-negative bacteria, *gyrA* mutations are dominant mutations in *E. coli (6)*. Consequently, in this study, all isolates (59/59 isolates; 100%) had *gyrA* mutations, and most isolates (58/59 isolates; 98.3%) had *parC* mutations. All mutants that had *parC* and *parE* mutations also had *gyrA* mutations. These data supported previous studies

showing that *gyrA* mutations are a prerequisite for subsequent mutations in gram-negative bacteria *(11, 38)*. Among *gyrA* mutant isolates, the Ser83Leu (59/59 isolates; 100%) amino acid substitution was the most frequent mutation, whereas that in *parC* mutants was the Ser80Ile (56/58 isolates; 96.6%) mutation. This result is consistent with that observed in *E. coli* isolates from humans *(8, 30)*. Twenty-two *parE* mutant patterns were distributed widely (Ile355Thr, Leu416Phe, Ser458Ala, Glu460Lys, and Ile464Phe), and no isolates exhibited double mutations in *parE* compared with *gyrA* and *parC* patterns. In terms of the CIP MIC, all isolates with the *parE* mutation had MICs ranging from 16 to 256 μg/mL. These data indicate that bacteria may need the *parE* mutation to obtain a higher level of resistance to FQ.

FQ-resistant *E. coli* tolerate the drug through the production of plasmid-mediated genes. The possibility of conjugation with FQ-susceptible bacteria emphasizes the importance of PMQR genes *(31)*. To date, PMQR genes have been identified as the *qnr* family, which protects the gyrase from FQ, the modifying enzyme *aac(6')-Ib-cr* (cr variant of *aac(6')-Ib*), and the *qepA* gene encoding the efflux pump *(38)*. Bacteria usually require at least double mutations in QRDRs to acquire FQ resistance *(22)*. However, in this study, one isolate (CIP MIC: 4 μg/mL) only had a single mutation in *gyrA* and harbored *qnrS* in its

plasmid. This result is consistent with the function of PMQR, contributing to FQ resistance *(12)*.

In this study, the overall prevalence of PMQR in FQ-resistant *E. coli* from pigs was considerably higher than that in a previous study in Korea (4.3% versus 15.3%) *(26)*. Notably, these findings indicated that the spread of PMQR genes had increased in pig farms and that the risk of PMQR spread in livestock was considerable. The prevalent PMQR gene found in this study was different from that in human isolates in Korea *(27)*. The predominant PMQR genes detected in human isolates were *qnrA* and *qnrB*, whereas *qnrS* was the prevalent PMQR in this study, indicating that the origin of PMQR was different between humans and pigs in Korea.

Antimicrobial susceptibility tests against an additional seven classes of drugs (a total of 16 antimicrobial agents), including quinolone (NA) and FQ (CIP) were performed to detect ESBL-producing *E. coli* and MDR isolates. These findings supported previous results showing the high prevalence of MDR isolates in FQ-resistant bacteria *(25)*. A study by Karlowsky et al. *(14)* found that as the CIP MIC increases, the rates of resistance to ampicillin, cefdinir, and nitrofurantoin also increase. When FQ-resistant isolates were divided in two groups based on MICs (Table 6), similar to a study by Karlowsky et al. *(14)*, MDR and ESBL *E. coli* were detected significantly more in high CIP MIC group

than in low CIP MIC group, respectively. These findings suggest that high FQ-resistance is strongly related to the emergence of MDR and ESBL *E. coli*.

In this study, a correlation between CIP MICs and efflux pump activity was not found. Of 59 CIP-resistant *E. coli*, 56 isolates (94.9%) had efflux pump activity based on OST results. Interestingly, isolates 4 and 5 had the same MICs, QRDR mutations, and PMQR results, but had divergent OST results. These isolates already had double mutations in *gyrA* and a single mutation in *parC*. This finding is consistent with that of a previous study showing that efflux pump activity usually does not further affect the MIC in bacteria having high levels of resistance to FQ owing to target alteration *(1)*. Indeed, the low R-value (0.044) found by the Pearson correlation coefficient method verified this assertion. Target modification is the most important mechanism through which *E. coli* reaches the FQ-resistance breakpoint. Additionally, the presence of PMQR genes can support FQ resistance through the functions of the encoded proteins. However, when *E. coli* exhibits high levels of resistance, efflux pump activity is not an important factor.

In conclusion, the effort to reduce the use of FQ in food animals was not successful. Prohibition of FQ as a feed additive has led to alterations in FQ use for therapeutic and self-treatment purposes without substantial changes in the total amount of FQ use in the food animal industry in Korea *(21)*. Consequently,

the prevalence of FQ-resistant and MDR *E. coli* is considerably high in the Korean swine industry. Moreover, compared with a previous study in Korea *(26)*, the current study showed that there was a considerable increase in PMQR prevalence in food-producing animals. These FQ-resistant bacteria from food-producing animals usually have additional resistance to other types of antimicrobials, and the mechanisms are typically similar to those of human clinical isolates *(11)*. When these resistant bacteria are transmitted to the human community, they could be serious threats to public health. Accordingly, considering the clinical importance of FQ in veterinary and human medicine, prescriptions and other uses of FQ should be carefully monitored and regulated, in conjunction with the ban of FQ use as a feed additive, in order to reduce FQ resistance in food-producing animals.

Table 1. Swine fecal samples investigated in this study

Province	No. of farms	No. of swine	type of swine					
			Piglet[a]	Gilt	Sow	Growing	Porker	Unknown
Jeju	5	86	29	9	17	17	14	
Jeolla	7	82	18		17	9	7	31
Gyeongsang	5	35	5		1	3		26
Chungcheong	4	24			1			23
Gyeonggi	2	6					1	5
Gangwon	1	4						4
Total	24	237	52	9	36	29	22	89

[a]Terminology: Piglet, a young pig; Gilt, female pig which has not had piglets; Sow, female pig which has had piglets; Growing, a pig grown for porker; Porker, a pig grown to pork.

Table 2. Polymerase chain reaction primers for detecting QRDR mutations and PMQR

	Primers	Sequence (5′→3′)	Size (bp)	Reference
***E. coli* Identification**	*E. coli* 16S rRNA	F: GGGAGTAAAGTTAATACCTTTGCTC R1: TTCCCGAAGGCACATTCT R2: TTCCCGAAGGCACCAATC	584	*(28)*
QRDR[a]	*gyrA*	F: TACACCGGTCAACATTGAGG R: TTAATGATTGCCGCCGTCGG	648	*(9)*
	gyrB	F: GAAATGACCCGCCGTAAA R: ACGACCGATACCACAGCC	272	*(10)*
	parC	F: AAACCTGTTCAGCGCCGCATT R: GTGGTGCCGTTAAGCAAA	395	*(10)*
	parE	F: CTGACCGAAAGCTACGTCAACC R: CGTTCGGCTTGCCTTTCTTG	892	*(4)*
PMQR[b]	*qnrA*	F: CAACTTGAGTGGCCAATGCC R: GACTCCTTCGAGGTTGACCC	211	This study
	qnrB	F: GGMATHGAAATTCGCCACTG R: TTTGCYGYYCGCCAGTCGAA	264	*(7)*
	qnrS	F: ACGACATTCGTCAACTGCAA R: TAAATTGGCACCCTGTAGGC	417	*(36)*
	qepA	F: GCAGGTCCAGCAGCGGGTAG R: CTTCCTGCCCGAGTATCGTG	199	*(35)*
	aac(6')-Ib	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	482	*(36)*

[a]QRDR: Quinolone resistance-determining region.

[b]PMQR: Plasmid-mediated quinolone-resistance.

Table 3. Fluoroquinolone antimicrobial resistance rate of *E. coli* from swine farms at different province

Province	**No. of *E. coli***	**No. of FQ resistant *E. coli* (%)**
Jeju	48	6 (12.5%)
Jeolla	73	33 (45.2%)
Gyeongsang	19	8 (42.1%)
Chungcheong	22	11 (50.0%)
Gyeonggi	6	1 (16.7%)
Gangwon	3	0 (0.0%)
Total	171	59 (34.5%)

Table 4. Proportions of multidrug resistance and ESBL-producing *E. coli*

FQ-resistant isolates	Number of drug class resistances	Antimicrobial agents										ESBL
		AM[a]	GM	CTX	AMC	ATM	C	CRO	CTT	SXT	TE	
Non-MDR	1 (n = 5)	0	0	0	0	0	0	0	0	0	0	0
MDR	3 (n = 7)	7	2	0	1	0	0	0	0	0	5	0
	4 (n = 4)	4	1	0	1	0	3	0	0	2	2	0
	5 (n = 18)	18	10	2	3	1	15	2	0	13	14	2
	6 (n = 21)	21	17	3	2	2	21	4	1	21	20	5
	7 (n = 4)	4	3	2	1	0	3	4	1	4	4	4
Total (n = 59, %)		54 (91.5%)[c]	33 (55.9%)	7 (11.9%)	8 (13.6%)	3 (5.1%)	42 (71.2%)	10 (16.9%)	2 (3.4%)	40 (67.8%)	45 (76.3%)	11 (18.6%)[b]

[a]Abbreviations: AM, ampicillin; GM, gentamicin; CTX, cefotaxime; AMC, amoxicillin-clavulanic acid; ATM, aztreonam; C, chloramphenicol; CRO, ceftriaxone; CTT, cefotetan; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline.

[b]% of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* among the total of ESBL positive isolates.

[c]% of total resistance to each drug agent.

Table 5. Amino acid substitutions in the QRDRs and prevalence of PMQR genes of 59 FQ-resistant *E. coli* isolates

MIC (μg/ml)		QRDR mutations			Isolate number	PMQR
NA	**CIP**	***gyrA***	***parC***	***parE***		
8	0.008				ATCC 25922	
256	4	S83L			58[a]	*qnrS*
>1024	8	S83L	S80I		1	*qnrS*
		S83L/D87N	S80I		4, 5, 26, 30, 31, 37, 46, 53, 59	
				E84K	42	
				A56T/S80I	8, 17, 28	
		S83L/D87G	S80I		15, 16, 18, 19, 43, 45	
		S83L/D87H	S80I		22, 44	
>1024	16	S83L/D87N	S80I		56, 57	
			A56T/S80I		29	
			S80R	I464F	27	
		S83L/D87G	S80I		14	
>1024	32	S83L/D87N	S80I	L416F	6	
			S80I		38	
			S80I	S458A	2,	
				S458A	7	*qnrS*
			S80I	I355T/S458A	13[a]	
			A56T/S80I		50	*qnrS*
			S80I/E84A		34, 36, 40, 41	
			S80I/E84A	I464F	35	
		S83L/D87Y	S80I	S458A	33[a]	
>1024	64	S83L/D87N	S80I	S458A	10[a], 21, 39	*qnrS*
					20	
			S80I		25	*qepA*
			S80I	E460K	51, 52, 54	
			S80I/E84G		24[a]	
		S83L/D87Y	S80I	S458A	3, 32[a]	
			S80I/E84G	I355T	9 [a], 11 [a], 12 [a], 23[a]	
>1024	128	S83L/D87N	S80I/E84G		55	
>1024	256	S83L/D87N	S80I	S458A	47[a], 48	
			S80I/E84G		49	*aac(6')-Ib-cr*
Total number of isolates					**59 (11)[b]**	**9**

[a]Extended-spectrum beta-lactamase (ESBL)-producing *E. coli*.

[b]The number in the parenthesis indicates the total number of ESBL-producing *E. coli*.

Table 6. Proportions of multidrug resistance and ESBL-producing *E. coli* at different minimum inhibitory concentration (MIC) groups

Group	CIP MIC (μg/mL)	Number of MDR	Number of ESBL
Low MIC group (n=28)	≤16	23 (82.1%)[a]	1 (3.6%)[b]
High MIC group (n=31)	>16	31 (100%)	10 (32.3%)
Total (n=59)		54	11

[a]Percentage of multidrug resistance (MDR) or extended-spectrum beta-lactamase (ESBL) in each group.

REFERENCES

1. Aathithan, S., and G. French. 2009. Organic solvent tolerance and fluoroquinolone resistance in *Klebsiella pneumoniae* clinical isolates. *Journal of antimicrobial chemotherapy*. 64:870-871.
2. Aldred, K. J., R. J. Kerns, and N. Osheroff. 2014. Mechanism of quinolone action and resistance. *Biochemistry*. 53:1565-1574.
3. Andrews, J. 2012. Detection of extended-spectrum beta-lactamases (ESBLs) in *E. coli* and *Klebsiella* species. *Brit. Soc. Antimicrobial. Chemotherapy.*, *http://bsac. org. uk/wp-content/uploads/2012/02/Ecoliklebsiella. pdf*.
4. 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. *Annals of microbiology*. 62:293-298.
5. Beutin, L., S. Zimmermann, and K. Gleier. 1996. Rapid detection and isolation of shiga-like toxin (verocytotoxin)-producing *Escherichia coli* by direct testing of individual enterohemolytic colonies from washed sheep blood agar plates in the VTEC-RPLA assay. *Journal of Clinical Microbiology*. 34:2812-2814.
6. Bolon, M. K. 2011. The newer fluoroquinolones. *Medical Clinics of North America*. 95:793-817.
7. Cattoir, V., L. Poirel, V. Rotimi, C.-J. Soussy, and P. Nordmann. 2007.

Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *Journal of Antimicrobial Chemotherapy*. 60:394-397.

8. Domínguez, E., M. Zarazaga, Y. Sáenz, L. Briñas, and C. Torres. 2002. Mechanisms of antibiotic resistance in *Escherichia coli* isolates obtained from healthy children in Spain. *Microbial Drug Resistance*. 8:321-327.
9. Dutta, S., Y. Kawamura, T. Ezaki, G. B. Nair, K.-I. Iida, and S.-I. Yoshida. 2005. Alteration in the g*yrA* subunit of DNA gyrase and the *parC* subunit of topoisomerase IV in quinolone-resistant Shigella dysenteriae serotype 1 clinical isolates from Kolkata, India. *Antimicrobial agents and chemotherapy*. 49:1660-1661.
10. Fendukly, F., I. Karlsson, H. Hanson, G. Kronvall, and K. Dornbusch. 2003. Patterns of mutations in target genes in septicemia isolates of *Escherichia coli* and *Klebsiella pneumoniae* with resistance or reduced susceptibility to ciprofloxacin. *Apmis*. 111:857-866.
11. Hopkins, K. L., R. H. Davies, and E. J. Threlfall. 2005. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *Int J Antimicrob Agents*. 25:358-73.
12. Jacoby, G. A. 2005. Mechanisms of resistance to quinolones. *Clinical Infectious Diseases*. 41:S120-S126.

13. Karczmarczyk, M., M. Martins, T. Quinn, N. Leonard, and S. Fanning. 2011. Mechanisms of fluoroquinolone resistance in *Escherichia coli* isolates from food-producing animals. *Applied and environmental microbiology*. 77:7113-7120.

14. Karlowsky, J. A., D. J. Hoban, M. R. DeCorby, N. M. Laing, and G. G. Zhanel. 2006. Fluoroquinolone-resistant urinary isolates of *Escherichia coli* from outpatients are frequently multidrug resistant: results from the North American Urinary Tract Infection Collaborative Alliance-Quinolone Resistance study. *Antimicrobial agents and chemotherapy*. 50:2251-2254.

15. Kim, J.-C., L. Chui, Y. Wang, J. Shen, and B. Jeon. 2016. Expansion of Shiga Toxin–Producing *Escherichia coli* by Use of Bovine Antibiotic Growth Promoters. *Emerging infectious diseases*. 22:802.

16. Legates, D. R., and G. J. McCabe. 1999. Evaluating the use of "goodness-of-fit" measures in hydrologic and hydroclimatic model validation. *Water resources research*. 35:233-241.

17. Lim, S.-K., H.-S. Lee, H.-M. Nam, Y.-S. Cho, J.-M. Kim, S.-W. Song, Y.-H. Park, and S.-C. Jung. 2007. Antimicrobial resistance observed in *Escherichia coli* strains isolated from fecal samples of cattle and pigs in Korea during 2003–2004. *International journal of food microbiology*. 116:283-286.

18. Lim, S.-K., J.-E. Lee, H.-S. Lee, H.-M. Nam, D.-C. Moon, G.-C. Jang, Y.-J.

Park, Y.-G. Jung, S.-C. Jung, and S.-H. Wee. 2014. Trends in antimicrobial sales for livestock and fisheries in Korea during 2003-2012. *Korean Journal of Veterinary Research*. 54.

19. Organization, W. H. 2005. Critically important antibacterial agents for human medicine for risk management strategies of non-human use: report of a WHO working group consultation, 15-18 February 2005, Canberra, Australia.
20. Park, C. H., A. Robicsek, G. A. Jacoby, D. Sahm, and D. C. Hooper. 2006. Prevalence in the United States of *aac(6')-Ib-cr* encoding a ciprofloxacin-modifying enzyme. *Antimicrob Agents Chemother*. 50:3953-5.
21. QIA. 2015. Monitoring of national antimicrobials resistance in Korean livestock industry. p. 1-115. *In* A.a.p.q. agency (ed.) Animal and plant quarantine agency, Animal and plant quarantine agency.
22. Ruiz, J. 2003. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J Antimicrob Chemother*. 51:1109-17.
23. Sayah, R. S., J. B. Kaneene, Y. Johnson, and R. Miller. 2005. Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic-and wild-animal fecal samples, human septage, and surface water. *Applied and environmental microbiology*. 71:1394-1404.
24. Smith, P., K. Tomfohrde, D. Rhoden, and A. Balows. 1972. API system: a

multitube micromethod for identification of Enterobacteriaceae. *Applied microbiology*. 24:449.

25. Strand, L., A. Jenkins, I. H. Henriksen, A. G. Allum, N. Grude, and B. E. Kristiansen. 2014. High levels of multiresistance in quinolone resistant urinary tract isolates of *Escherichia coli* from Norway; a non clonal phenomen? *BMC research notes*. 7:376.
26. Tamang, M. D., H. M. Nam, M. H. Chae, S. R. Kim, M. Gurung, G. C. Jang, S. C. Jung, and S. K. Lim. 2012. Prevalence of plasmid-mediated quinolone resistance determinants among *Escherichia coli* isolated from food animals in Korea. *Foodborne Pathog Dis*. 9:1057-63.
27. Tamang, M. D., S. Y. Seol, J. Y. Oh, H. Y. Kang, J. C. Lee, Y. C. Lee, D. T. Cho, and J. Kim. 2008. Plasmid-mediated quinolone resistance determinants *qnrA*, *qnrB*, and *qnrS* among clinical isolates of Enterobacteriaceae in a Korean hospital. *Antimicrob Agents Chemother*. 52:4159-62.
28. Tsen, H., C. Lin, and W. Chi. 1998. Development and use of 16S rRNA gene targeted PCR primers for the identification of *Escherichia coli* cells in water. *Journal of applied microbiology*. 85:554-560.
29. Van den Bogaard, A., N. London, and E. Stobberingh. 2000. Antimicrobial resistance in pig faecal samples from The Netherlands (five abattoirs) and Sweden. *Journal of Antimicrobial Chemotherapy*. 45:663-671.

30. Vila, J., J. Ruiz, F. Marco, A. Barcelo, P. Goni, E. Giralt, and T. J. De Anta. 1994. Association between double mutation in *gyrA* gene of ciprofloxacin-resistant clinical isolates of *Escherichia coli* and MICs. *Antimicrobial agents and chemotherapy*. 38:2477-2479.

31. Wang, M., D. F. Sahm, G. A. Jacoby, and D. C. Hooper. 2004. Emerging plasmid-mediated quinolone resistance associated with the *qnr* gene in Klebsiella pneumoniae clinical isolates in the United States. *Antimicrobial agents and chemotherapy*. 48:1295-1299.

32. White, D. G., J. D. Goldman, B. Demple, and S. B. Levy. 1997. Role of the acrAB locus in organic solvent tolerance mediated by expression of *marA*, *soxS*, or *robA* in *Escherichia coli*. *Journal of Bacteriology*. 179:6122-6126.

33. Wikler, M. A., and F. R. Cockerill. 2008. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement. Wayne: Clinical and Laboratory Standards Institute*.

34. Witte, W. 1998. Medical consequences of antibiotic use in agriculture. *Science*. 279:996-997.

35. Yamane, K., J.-i. Wachino, S. Suzuki, and Y. Arakawa. 2008. Plasmid-mediated *qepA* gene among *Escherichia coli* clinical isolates from Japan. *Antimicrobial agents and chemotherapy*. 52:1564-1566.

36. Yue, L., H.-X. Jiang, X.-P. Liao, J.-H. Liu, S.-J. Li, X.-Y. Chen, C.-X. Chen, D.-H. Lü, and Y.-H. Liu. 2008. Prevalence of plasmid-mediated quinolone resistance *qnr* genes in poultry and swine clinical isolates of *Escherichia coli*. *Veterinary microbiology*. 132:414-420.

37. Yue, L., H. X. Jiang, X. P. Liao, J. H. Liu, S. J. Li, X. Y. Chen, C. X. Chen, D. H. Lu, and Y. H. Liu. 2008. Prevalence of plasmid-mediated quinolone resistance *qnr* genes in poultry and swine clinical isolates of *Escherichia coli*. *Vet Microbiol*. 132:414-20.

38. Zurfluh, K., H. Abgottspon, H. Hachler, M. Nuesch-Inderbinen, and R. Stephan. 2014. Quinolone resistance mechanisms among extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* isolated from rivers and lakes in Switzerland. *PLoS One*. 9:e95864.

국문초록

국내 돼지 분변 유래 대장균 분리주의 플루오로퀴놀론 항생제 내성 연구

서울대학교 대학원
수의학과 수의미생물학전공
허 윤 성
(지도교수: 박 용 호)

플루오로퀴놀론 계열 항생제는 사람과 동물 모두에서 중요한 항생제 이다. 또한, 플루오로퀴놀론 내성 세균은 항생제 다제내성균과 깊은 연관이 있는 것으로 많은 선행 연구를 통해 밝혀졌다. 따라서 본 연구에서는 국내 돼지 분변에서 분리된 대장균을 통해 플루오로퀴놀론 내성 패턴 및 기전을 확인하고자 하였다. 전국 24 개 농장, 총 237 개의 돼지 분변 스왑 샘플에서 171 주 (72.2%)의 대장균을 분리 하였고, CLSI 에 기준한 항생제 디스크 검사를 통해 59 주 (34.5%)의 플루오로퀴놀론 내성 대장균을 분리 하였다. 본 연구에서는 플루오로퀴놀론 내성 기전을 확인하기 위하여 총 3 가지의 내성 기전을 연구하였다 (i. QRDR 의 아미노산 돌연변이, ii. 플라스미드 내성 유전자, iii. Efflux pump 기전). 플루오로퀴놀론 내성

기전에서 가장 중요한 역할을 한다고 알려진 gyrase 와 topoisomerase IV 의 아미노산 돌연변이는 모든 균주에서 *gyrA* 유전자의 돌연변이가 발견되었다. 그 다음으로 *parC* (58 주, 98.3%), *parE* (22 주, 37.3%) 유전자에서 아미노산 돌연변이가 발견되었다. 하지만 *gyrB* 유전자에서는 돌연변이가 발견되지 않았다. 다른 세균으로의 항생제 내성 유전자 전파가 가능하다고 알려진 플라스미드의 항생제 내성 유전자는 59 주의 균주중에서 총 9 주 (15.3%)에서 확인 되었으며, 이는 과거 국내에서의 연구보다 더 높은 수치였다. 가장 많은 균주에서 *qnrS* (7 주, 11.9%)가 발견되었으며, *qepA* (1 주, 1.7%), *aac(6')-Ib-cr* (1 주, 1.7%) 순으로 발견되었다. 다른 균주로의 항생제 내성 유전자를 전파시킬 수 있는 플라스미드 유전자가 과거 보다 더 많아졌다는 점에서 앞으로 집중적인 관리가 필요할 것이다. 총 59 주의 플루오로퀴놀론 내성 대장균에서 56 주 (94.9%)의 대장균이 efflux pump activity 가 관찰되었다. 우리나라에서는 항생제 내성 세균 관리를 위하여 축산용 항생제 사용에 대해 강력한 규제를 해오고 있다. 하지만 플루오로퀴놀론 같은 사람과 동물 모두에서 중요하게 사용되어지고 있는 항생제에 대한 더 많은 연구와 규제가 필요할 것이다.

주요어: quinolone, fluoroquinolone, antimicrobial resistance, *Escherichia coli*, pig

학 번 : 2015-21825