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RESEARCH ARTICLE

Fluoroquinolone Resistance and Genetic Determinants of *Escherichia coli* Isolated from Conventional and Integrated Swine Farms in Korea

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ABSTRACT

Emergence of fluoroquinolone-resistant *Escherichia coli* in swine poses concerns for managing antimicrobial resistance. This study compared antimicrobial resistance profiles, molecular resistance determinants, and genetic relatedness of fluoroquinolone-resistant *E. coli* isolated from integrated and conventional swine farms in South Korea. A total of 130 *E. coli* isolates were obtained from fecal samples collected from 26 farms (13 integrated and 13 conventional farms). Antimicrobial susceptibility testing was performed using disc diffusion and broth microdilution methods. Fluoroquinolone-resistant isolates were analyzed for quinolone resistance-determining region (QRDR) mutations, plasmid-mediated quinolone resistance (PMQR) genes, and multi-locus sequence typing (MLST). Isolates from the conventional farms showed significantly higher resistance rates to several antimicrobials than those from the integrated farms. Among the 58 fluoroquinolone-resistant isolates, QRDR mutations in *gyrA* and *parC* were predominant in all samples, with the *gyrA* S83L/D87N and *parC* S80I mutations being the most frequently detected. PMQR gene frequencies were comparable in both farm system types and were distributed across multiple sequence types (STs). Fluoroquinolone minimum inhibitory concentrations (MICs) were generally high in both groups; whereas enrofloxacin MICs tended to be higher among the isolates from the conventional farms. MLST-based minimum spanning tree analysis revealed that resistant isolates from both farm types were distributed across multiple STs with limited allelic variation rather than being confined to farm-specific lineages. These findings indicated that, although farm management systems influenced the prevalence of antimicrobial resistance, fluoroquinolone resistance in swine-associated *E. coli* was maintained within a shared genetic background, emphasizing the need for integrated resistance management strategies beyond antimicrobial usage alone.

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INTRODUCTION

The emergence and spread of antimicrobial-resistant bacteria resulting from antimicrobial use are widely recognized as major global public health concerns. In the livestock sector, antimicrobial use has been identified as a key factor in accelerating the selection and accumulation of resistant bacteria, particularly among commensal intestinal microorganisms (Guo *et al.*, 2021; Khadse *et al.,* 2023; Toya *et al.*, 2025). The swine industry, which is characterized by intensive production systems and repeated antimicrobial exposure, has been highlighted as a sector with a high risk of developing and spreading antimicrobial resistance (Abdalla *et al.*, 2021; Jaleta *et al.*, 2024; Mencía-Ares *et al.*, 2021; Oliveira *et al.*, 2024).

Escherichia coli is a predominant commensal bacterium inhabiting the gastrointestinal tract of pigs and has been extensively utilized as an indicator organism in antimicrobial resistance surveillance studies (Toya *et al.*, 2025). Among antimicrobials, fluoroquinolones are classified as critically important agents for treating severe infections in both humans and animals, and increasing

resistance to this class of drugs has raised substantial clinical and epidemiological concerns (WHO, 2018). Fluoroquinolone resistance in *E. coli* primarily arises from mutations in the quinolone resistance-determining regions (QRDRs) of the DNA gyrase (*gyrA*) and topoisomerase IV (*parC*) genes, and plasmid-mediated quinolone resistance (PMQR) genes contribute to the maintenance and horizontal spread of fluoroquinolone resistance (Seo *et al*., 2022).

Previous studies of fluoroquinolone-resistant *E. coli* isolated from swine farms have mainly focused on specific production stages, host populations, or restricted rearing environments, and have provided fundamental insights into the molecular mechanisms of fluoroquinolone resistance and the clonal distribution of resistant strains (Cheng *et al.*, 2020; Koster *et al.*, 2023; Do *et al.,* 2025a). However, comparative evaluation of the influence of farm management systems on the selection and persistence of fluoroquinolone-resistant *E. coli* remains limited.

Integrated production systems are characterized by systematic coordination and management of breeding, weaning, growing, and finishing stages within a unified production framework, with animal movement and production flow governed by predefined management protocols (Seo *et al.*, 2023; Do *et al.*, 2025b). In contrast, conventional production systems are often associated with more fragmented management across production stages and more frequent introduction of external animals, resulting in more complex contact patterns between animals and their surrounding environment (Seo *et al.*, 2023; Do *et al.*, 2025b). In Korea, integrated swine production systems are generally characterized by higher levels of vertical integration, standardized biosecurity protocols, and centralized decision-making regarding antimicrobial use; whereas, conventional farms often rely on farm-level discretion with greater variability in antimicrobial administration practices (Seo *et al.*, 2023; Do *et al.*, 2025b). In addition, veterinary oversight and disease-control practices are often implemented through centralized management in integrated systems, whereas conventional farms generally rely more on farm-specific decision-making (Do *et al.*, 2025b). Such differences in management systems may influence antimicrobial usage practices, persistence of resistant bacteria, and transmission dynamics of antimicrobial-resistant microorganisms within farms (Do *et al.*, 2025b). However, studies that directly compare the molecular and phylogenetic characteristics of fluoroquinolone-resistant *E. coli* in integrated and conventional swine production systems are scarce.

Therefore, the objective of this study was to compare fluoroquinolone-resistant *E. coli* isolated from integrated and conventional swine farms by analyzing antimicrobial susceptibility profiles and fluoroquinolone minimum inhibitory concentrations (MICs), characterizing QRDR mutations by sequencing of the *gyrA* and *parC* genes, and assessing the genetic relatedness of PMQR-positive isolates using multi-locus sequence typing (MLST). This study aimed to provide fundamental insights into the ways in which differences in swine farm management systems influence the distribution and characteristics of fluoroquinolone-resistant *E. coli*, thereby contributing to the development of effective antimicrobial resistance management strategies for swine production.

MATERIALS AND METHODS

Bacterial isolates: Fecal samples were collected from 26 swine farms across different regions of South Korea from 2021 to 2022, comprising 13 farms operating under an integrated system and 13 farms operating under a conventional system. At each farm, fecal samples (approximately 10g) were collected from 15 different locations using sterile gauze swabs, and were transported to the laboratory under chilled conditions within 24h. Each sample was streaked onto MacConkey agar (BD Biosciences) and incubated at 37°C for approximately 12h. Presumptive *E. coli* colonies exhibiting typical pink pigmentation were then selected and confirmed by PCR as previously described (Do *et al.,* 2025a). For standardization, five *E. coli* isolates originating from different sampling locations were randomly selected from each farm. An equal number of isolates was included from each farm to avoid overrepresentation of individual farms and to facilitate direct comparisons between farm management systems. Consequently, 65 isolates were obtained from integrated farms and 65 isolates were obtained from conventional farms, yielding 130 *E. coli* isolates. All isolates were stored at −70°C until further analysis.

Antimicrobial susceptibility testing: Antimicrobial susceptibilities of all the *E. coli* isolates were determined according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. The following antimicrobial agents were evaluated: ampicillin (10μg), amoxicillin–clavulanic acid (20/10μg), piperacillin-tazobactam (100/10μg), cefazolin (30μg), cefuroxime (30μg), cefoxitin (30μg), ceftiofur (30μg), cefotaxime (30μg), ceftazidime (30μg), cefepime (30μg), gentamicin (10μg), streptomycin (10μg), kanamycin (30μg), oxytetracycline (30μg), tetracycline (30μg), tigecycline (15μg), florfenicol (30μg), chloramphenicol (30μg), nalidixic acid (30μg), ciprofloxacin (5μg), sulfisoxazole (250μg), trimethoprim–sulfamethoxazole (1.25/23.75μg), and meropenem (10μg). The inhibition zone diameters were interpreted according to the CLSI criteria. The MICs of ciprofloxacin, norfloxacin, and enrofloxacin were determined using the broth microdilution method, following the CLSI guidelines. *E. coli* ATCC 25922 was used as a quality control strain for disc diffusion and MIC testing.

Molecular analysis: PCR was performed to amplify the QRDR of *gyrA* and *parC* to identify mutations in the 58 fluoroquinolone-resistant *E. coli* isolates (34 isolates from conventional farms and 24 isolates from integrated farms), as previously described (Do *et al.,* 2025a). The PCR amplicons of *gyrA* and *parC* were sequenced, and similarity was assessed using BLAST. The PMQR genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6')-Ib-cr*, and *qepA*) were identified by PCR and sequencing. The PMQR-positive isolates were further screened by PCR for β-lactamase-associated resistance genes (*blaTEM*, *blaSHV*, *blaOXA*, and *blaCTX-M* family), tetracycline (*tetA*, *tetB*, and *tetC*), chloramphenicol (*cmlA* and *catA1*), sulfonamide (*sul1* and *sul2*), and aminoglycosides (*aac(3)-II* and *ant(2")-I*). In addition, class I and II integrons (*intl1* and *intl2*) were examined by PCR, and gene cassettes in integron-positive isolates were characterized by sequencing.

Multi-locus sequence typing and minimum spanning tree (MST) analysis: MLST was performed for all PMQR-positive *E. coli* isolates based on the sequence analysis of seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA,* and *recA*) according to the Achtman scheme (Wirth *et al.*, 2006). Allelic profiles and sequence types (STs) were assigned using the *E. coli* MLST database available at PubMLST (https://pubmlst.org).

To assess clonal relationships among isolates, an MST was constructed using the MLST allele profiles. Pairwise allelic distances were calculated as the number of loci with discordant allele numbers across the seven MLST loci. A complete weighted graph was generated from the resulting distance matrix, and an MST was inferred using igraph (version 2.1.1) in R (version 4.4.1). The MST was visualized in RStudio (version 2024.12.0+467), with nodes annotated according to the farm management system (integrated vs. conventional) and PMQR status.

Statistical analysis: All statistical analyses were performed using IBM SPSS Statistics (version 27.0; IBM Corp., Armonk, NY, USA). Differences in antimicrobial resistance rates between the farm types were evaluated using chi-square tests. Statistical significance was set at $P<0.05$.

RESULTS

Antimicrobial resistance: A comparison of the antimicrobial resistance patterns of *E. coli* isolated from integrated and conventional farms revealed significant differences between the farm types for certain antimicrobials (Fig. 1). The resistance rate to piperacillin–tazobactam (i.e., a β-lactam/β-lactamase inhibitor combination) was 1.5% (1/65) in strains from integrated farms and 23.1% (15/65) in strains from conventional farms, indicating a significantly higher resistance rate in conventional farms. Furthermore, a significantly higher resistance rate to cefazolin (i.e., a first-generation cephalosporin) was observed in samples from conventional farms (87.7%, 57/65) than in those from integrated farms (69.2%, 45/65). Among the third-generation cephalosporins, the resistance rates to both ceftiofur and ceftazidime were significantly higher in the strains from the conventional farms than in those from the integrated farms. Tigecycline resistance was not observed in the strains from the integrated farms (0.0%, 0/65), but was detected in 21.5% (14/65) of the strains from the conventional farms. Although not statistically significant, compared to those observed in the strains from the integrated farms, higher resistance rates were observed in the strains from the conventional farms for amoxicillin–clavulanate, cefuroxime, cefoxitin, cefotaxime, cefepime, gentamicin, streptomycin, kanamycin, florfenicol, chloramphenicol, nalidixic acid, ciprofloxacin, and sulfisoxazole. Meropenem resistance was not observed in strains from either farm type.

QRDR mutations, PMQR gene distribution, and fluoroquinolone MIC profiles: Fluoroquinolone-resistant *E. coli* isolates (n = 58; 34 from conventional farms and 24 from integrated farms) were analyzed to determine the PMQR gene distribution; QRDR mutations in *gyrA* and *parC* genes; and ciprofloxacin, enrofloxacin, and norfloxacin MICs (Table 1).

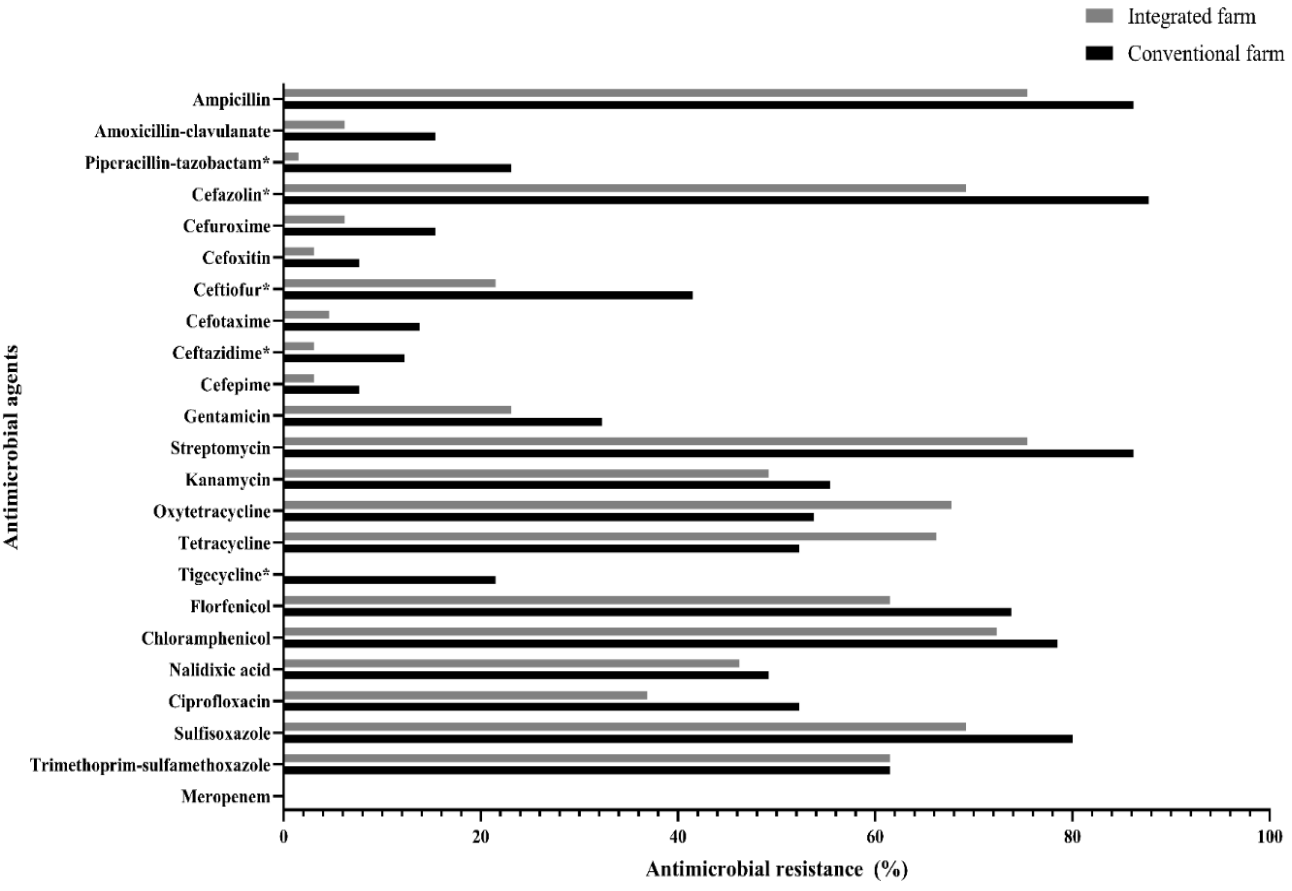

Fig. 1: Antimicrobial resistance profiles of *E. coli* from integrated and conventional swine farms in Korea.

Table 1: QRDR mutations and PMQR gene prevalence in 58 fluoroquinolone-resistant *E. coli* isolates from integrated and conventional swine farms in Korea

Farm type	Isolates	PMQR gene[1)]	Amino acid change[2)]		MIC (μg/mL)[3)]		
			gyrA	*parC*	CIP	ENR	NOR
Conventional farm	0337	*qnrS*	D87N	S80I	32	256	256
	0403	*qnrS*	D87N	S80I	64	>256	256
	0255	*qnrS*	S83L / D87N	WT	> 256	>256	256
	0301	*qnrS*	S83L / D87N	WT	8	8	0.5
	0309	*qnrS*	WT	WT	16	8	0.5
	0387	*qnrS*	WT	WT	16	16	1
	0412	*qnrD*	S83L / D87N	S80I	8	256	256
	0009	*qnrD*	S83L / D87N	S80I	256	256	256
	0109	*qnrB*	S83L / D87N	S80I	128	>256	256
	0316	*qnrD:aac(6')-Ib-cr*	D87N	S80I	32	>256	256
	0197	-	S83L / D87 Y	S80I	256	256	256
	0052	-	S83L / D87N	S80I	8	128	8
	0081	-	S83L / D87N	S80I	32	256	256
	0127	-	S83L / D87N	S80I	16	>256	256
	0128	-	S83L / D87N	S80I	16	>256	256
	0154	-	S83L / D87N	S80I	2	256	256
	0179	-	S83L / D87N	S80I	32	>256	256
	0224	-	S83L / D87N	S80I	8	>256	256
	0268	-	S83L / D87N	S80I	16	>256	256
	0272	-	S83L / D87N	S80I	64	>256	256
	0295	-	S83L / D87N	S80I	32	>256	256
	0303	-	S83L / D87N	S80I	16	256	256
	0024	-	S83L / D87N	E84K	> 256	128	256
	0036	-	S83L / D87N	S80I	256	128	256
	0152	-	S83L / D87N	S80I	256	256	256
	0416	-	S83L / D87N	S80I	256	256	256
	1220	-	S83L / D87N	S80R	256	128	256
	1287	-	S83L / D87N	S80I	256	128	8
	0125	-	S83L / D87 G	S80R	64	32	2
	0060	-	S83 * / D87N	S80I	128	>256	256
	0274	-	S83 * / D87N	S80I	64	>256	256
	0078	-	D87N	S80I	16	256	256
	0247	-	D87N	S80I	16	256	256
	0390	-	D87N	S80I	128	>256	256
Integrated farm	0575	*qnrD*	S83L / D87N	S80I	16	256	256
	0691	*qnrD*	S83L / D87N	S80I	16	128	256
	0627	*qnrD*	S83L / D87N	S80I	32	128	256
	0689	*qnrD*	S83L / D87N	S80I	8	128	8
	0672	*qnrD*	D87N	WT	1	8	1
	0950	*qnrB*	WT	WT	32	8	0.5
	1345	*qnrS*	S83 *	WT	128	128	8
	0972	*qnrS:qepA*	S83L	WT	64	256	8
	0698	-	S83L / D87N	S80I	16	128	8
	0745	-	S83L / D87N	S80I	16	128	8
	0769	-	S83L / D87N	S80I	64	>256	256
	1023	-	S83L / D87N	S80 F	16	256	256
	0988	-	S83L / D87N	WT	32	256	256
	0603	-	S83L / D87N	S80I	256	128	8
	0653	-	S83L / D87N	S80I	256	128	256
	0701	-	S83L / D87N	S80I	256	256	256
	1007	-	S83L / D87N	WT	> 256	256	256
	1362	-	S83L / D87N	S80I	256	128	256
	0650	-	S83L / D87N	S80I	64	>256	256
	0995	-	S83L	S80F	16	128	256
	0642	-	S83 * / D87G	S80R	32	128	256
	1111	-	S87L	S80I	32	32	2
	0611	-	D87N	WT	256	128	8
	1091	-	WT	S80I	32	128	256

[1)] WT, wild type; [2)] -, not detected; [3)] MIC, minimum inhibitory concentration; CIP, ciprofloxacin; ENR, enrofloxacin; NOR, norfloxacin. For quality control, *E. coli* ATCC 25922 showed MIC values of CIP: < 0.125 μg/mL, ENR: < 0.125 μg/mL, NOR: 0.5 μg/mL.

The PMQR gene was detected in 10 (29.4%) of the 34 isolates from the conventional farms, specifically *qnrS* (6 isolates), *qnrD* (2 isolates), *qnrB* (1 isolate), and 1 isolate harboring both *qnrD* and *aac(6')-Ib-cr*. Among the 24 integrated farm isolates, PMQR genes were detected in 8 isolates (33.3%). In addition, *qnrD* (five isolates), *qnrB* (one isolate), *qnrS* (one isolate), and a co-carriage of *qnrS* and *qepA* (one isolate) were confirmed.

QRDR analysis revealed the *gyrA* S83L/D87N double mutation in 23 (67.6%) of 34 isolates from conventional farms, and the D87N single mutation in 6 (17.6%) isolates. For *parC*, the S80I mutation was identified in 27 isolates (79.4%) and the S80R mutation was detected in 2 isolates (5.9%). Isolates that maintained the wild-type sequences in both *gyrA* and *parC* were identified in two isolates (5.9%). The MICs for ciprofloxacin, enrofloxacin, and norfloxacin in these strains were 16μg/mL, 8–16μg/mL, and 0.5–1μg/mL, respectively. Among the 24 isolates originating from integrated farms, the *gyrA* S83L/D87N double mutation was identified in 15 isolates (62.5%), and the D87N single mutation was observed in 2 isolates (8.3%). The S80I mutation in *parC* was detected in 14 isolates (58.3%), and

S80F and S80R mutations were identified in 2 isolates (8.3%) and 1 isolate (4.2%), respectively. One isolate (4.2%) of the isolates retained the wild-type sequence in both *gyrA* and *parC*, whereas eight isolates (33.3%) retained the wild-type sequence in either *gyrA* or *parC*.

Fluoroquinolone MIC analysis showed MIC_{50} and MIC_{90} values for all isolates were 32, 256μg/mL for ciprofloxacin; 256, > 256μg/mL for enrofloxacin; and 256, 256μg/mL for norfloxacin. Analysis by farm type showed that the ciprofloxacin MIC_{50} and MIC_{90} values were identical (32 and 256μg/mL, respectively) in integrated and conventional farms. Similarly, norfloxacin MIC_{50} and MIC_{90} values were 256 and 256μg/mL, respectively, in both groups. In contrast, for enrofloxacin, the MIC_{50} and MIC_{90} values for isolates from integrated farms were 128 and 256μg/mL, respectively, whereas those for isolates from conventional farms were 256 and > 256μg/mL, respectively.

Antimicrobial resistance characteristics of PMQR-harboring *E. coli*: Table 2 summarizes the antimicrobial-resistance phenotypes and genetic characteristics of the 18 PMQR-carrying *E. coli* isolates from the conventional and integrated farms. Among these, 10 isolates (55.6%) originated from the conventional farms and 8 isolates (44.4%) originated from the integrated farms. MLST analysis revealed that the PMQR-carrying strains belonged to 12 distinct STs. Among these, ST5229 was the most prevalent, with six strains (33.3%); whereas ST1642 (2 strains), ST10, ST48, ST75, ST101, ST410, ST542, ST654, ST3014, ST5416, and ST7203 were each identified in a single strain. All PMQR-carrying strains exhibited ciprofloxacin resistance. Concurrent resistance to nalidixic acid (seven isolates from conventional farms and eight isolates from integrated farms) and trimethoprim–sulfamethoxazole (seven isolates from conventional farms and eight isolates from integrated farms) was commonly observed. Analysis of other antimicrobial resistance genes revealed that *blaTEM* was present in 16 isolates (88.9%). *blaCTX-M* family genes were identified in four isolates (22.2%) from conventional farms and in one isolate (5.6%) from integrated farms. Class I integrons were detected in seven isolates (38.9%), including five conventional farm-derived isolates (27.8%) and two integrated farm-derived isolates (11.1%). The detected gene cassette combinations included *aadA22*, *aadA12*, *aadA15*, *aadA2–linF*, *dfrA12–OrfF–aadA2*, and *dhfrXII–aadA2*.

MLST-based MST analysis: MLST-based MST analysis revealed that the fluoroquinolone-resistant *E. coli* isolates could be classified into 12 distinct STs (Fig. 2). On the MST, the majority of connections between STs showed limited allelic differences of 1–5, and a continuous linkage structure composed of STs with relatively close genetic distances was observed. ST5229, which contained the largest number of isolates, appeared as a mixed node and included isolates from both integrated and conventional farms. ST5229 was directly connected to ST101 via a single allelic difference. ST101 was connected to ST1642, which was adjacent to ST75 and ST654. Thus, a series of STs, including ST5229, ST101, and ST1642, were continuously linked while maintaining limited allelic differences. An additional linkage structure connecting ST48, ST10, ST7203, and ST542 was observed, with these STs showing 1–4 allelic differences. Within this linkage structure, isolates originating from both integrated and conventional farms were also observed to co-occur in adjacent nodes or the same node. Overall, MST analysis revealed that fluoroquinolone-resistant isolates from both integrated and conventional farms were not confined to specific STs but rather were distributed among genetically proximate STs.

Table 2: Phenotypes and genotypes of 18 PMQR harboring *E. coli* from integrated and conventional swine farms in Korea

Farm type	Isolate	MLST ST	PMQR genes	Resistance phenotypes[1)]	Other Resistance genes	Integron and gene cassettes
Conventional farm	0109	410	*qnrB*	AM, TZP, CZ, FOX, FUR, S, K, TGC, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, tetB, sul2*	-
	0316	3014	*qnrD:aac(6')-Ib-cr*	AM, CZ, CXM, FUR, CTX, CAZ, FEP, GM, S, K, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, bla$_{CTX-M-15}$, aac(6')-Ib-cr, aac(3)-II, tetA, sul1, sul2*	I (dfrA12-OrfF(gcuF)-aadA2, dhfrXII-aadA2)
	0337	48	*qnrS*	AM, CZ, S, FFC, C, NA, CIP,	*bla$_{TEM}$*	-
	0403	1642	*qnrS*	AM, CZ, FUR, S, K, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, tetA, sul2*	-
	0412	5229	*qnrD*	AM, CZ, FUR, GM, S, K, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, aac(3)-II, tetA, sul2, cmlA*	-
	0009	542	*qnrD*	AM, CZ, FUR, GM, S, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, aac(3)-II, tetA, sul1, sul2*	I (-)
	0255	1642	*qnrS*	AM, TZP, CZ, CXM, FUR, CTX, CAZ, FEP, GM, S, K, T, FFC, NA, CIP, ST, SXT	*bla$_{CTX-M-15}$, tetB*	I (aadA12, aadA15, aadA2-linF(lincosamide))
	0301	101	*qnrS*	AM, CZ, CXM, FUR, CTX, S, T, TE, FFC, C, CIP, SXT	*bla$_{TEM}$, bla$_{CTX-M-27}$, tetA, cmlA*	I (-)
	0309	5416	*qnrS*	AM, AMC, CZ, FUR, K, T, TE, FFC, C, CIP,	*bla$_{TEM}$, tetB, sul2*	-
	0387	75	*qnrS*	AM, CZ, CXM, FUR, CTX, CAZ, FEP, GM, S, K, FFC, C, CIP, ST, SXT	*bla$_{CTX-M-15}$, aac(3)-II*	I (aadA22, linF)
Integrated Farm	0575	5229	*qnrD*	AM, CZ, CXM, FUR, CTX, CAZ, FEP, GM, S, K, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, bla$_{CTX-M-15}$, aac(3)-II, sul2, cmlA*	I (aadA22)
	0691	5229	*qnrD*	AM, CZ, FUR, S, K, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, tetA, sul2, cmlA*	-
	0627	5229	*qnrD*	AM, CZ, GM, S, K, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, aac(3)-II, tetA, sul2, cmlA*	-
	0672	654	*qnrD*	AM, CZ, CXM, FUR, CTX, CAZ, FEP, S, FFC, C, NA, CIP, SXT	*bla$_{TEM}$, bla$_{CTX-M-15}$, cmlA*	I (-)
	0689	5229	*qnrD*	AM, CZ, FUR, CTX, S, K, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, tetA, sul2, cmlA*	-
	0972	5229	*qnrS:qepA*	AM, S, K, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, tetA, sul2, cmlA*	-
	0950	10	*qnrB*	AM, S, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, tetA, sul2, cmlA*	-
	1345	7203	*qnrS*	AM, CZ, FUR, S, T, TE, FFC, C, NA, CIP, ST, SXT	*bla$_{TEM}$, aac(3)-II, tetA, cmlA*	-

[1)]AM: ampicillin; AMC: amoxicillin-clavulanic acid; TZP: piperacillin-tazobactam; CZ: cefazolin; CXM: cefuroxime; FOX: cefoxitin; FUR: ceftiofur; CTX: cefotaxime; CAZ: ceftazidime; FEP: cefepime; GM: gentamicin; S: streptomycin; K: kanamycin; T: oxytetracycline; TE: tetracycline; TGC: tigecycline; FFC: florfenicol; C: chloramphenicol; NA: nalidixic acid; CIP: ciprofloxacin; ST: sulfisoxazole; SXT: trimethoprim-sulfamethoxazole

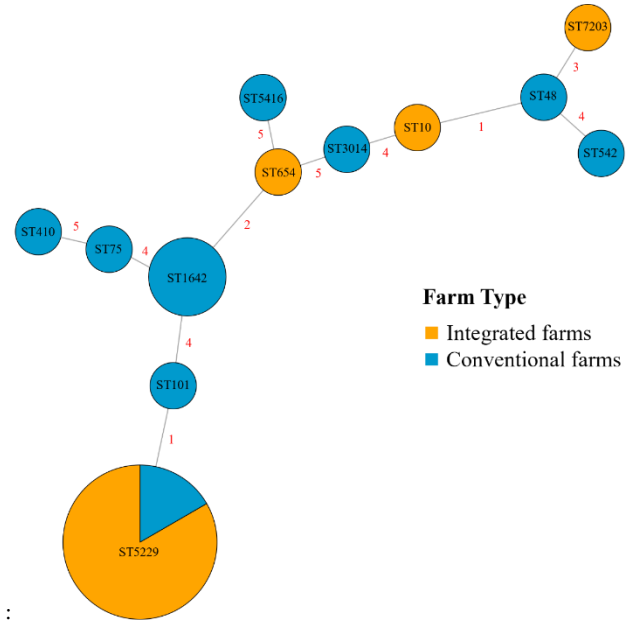

Fig. 2: MLST-based minimum spanning tree showing genetic relatedness among fluoroquinolone-resistant *E. coli* from integrated and conventional swine farms in Korea. Node size reflects the number of isolates per ST, and pie charts indicate the proportion of isolates from integrated (orange) and conventional (blue) farms. Numbers inside nodes denote STs, and numbers on edges indicate allelic differences across the seven MLST loci.

DISCUSSION

In this study, we comprehensively compared and analyzed the antimicrobial resistance patterns, QRDR mutations, PMQR gene distribution, and MLST-based genetic relationships of fluoroquinolone-resistant *E. coli* isolated from integrated and conventional swine farms in Korea. The results confirmed that differences in husbandry management systems influenced the resistance phenotypes and molecular resistance determinants, whereas fluoroquinolone-resistant strains were distributed across multiple STs showing limited allelic variation regardless of the farm type.

In this study, the *E. coli* strains originating from the conventional swine farms exhibited higher overall resistance rates to multiple antimicrobials, including β-lactam/β-lactamase inhibitor combinations and cephalosporins, than the strains from the integrated farms. This trend suggested that antimicrobial usage patterns and resistance selection pressures may vary depending on the farm management system, indicating a higher potential for accumulation of resistant bacteria, particularly in conventional farms where antimicrobial use is more decentralized and reliant on individual farm discretion. In Korea, integrated production systems generally operate under centralized veterinary oversight and standardized biosecurity programs, whereas conventional farms often implement these practices at the individual farm level, which may contribute to differences in antimicrobial resistance patterns (Do *et al.*, 2025b). Previous studies have indicated that farm management factors, including production structure and animal movement patterns, may influence cephalosporin and fluoroquinolone resistance rates in *E. coli* (Dohmen *et al.*, 2017; Gompel *et al.*, 2019; Harlow *et al.*, 2024). These findings align with the observed differences in resistance rates and directionality between the farm types in the present study. Notably, tigecycline resistance was detected exclusively in conventional farms in this study. Although tigecycline is an antimicrobial with limited use in livestock, several studies have reported cases in which resistance was observed alongside multidrug-resistant *E. coli* or extended-spectrum β-lactamase-producing strains in the environment without a direct history of its use (Dohmen *et al.*, 2017; Toya *et al.*, 2025). The overall trend of higher resistance in the strains from the conventional farms supported the notion that the farm management structure itself may influence the long-term accumulation of antimicrobial resistance. This finding demonstrated that factors other than the mere use of specific antimicrobials, including stocking density, animal movement, hygiene management, and the overall antimicrobial management system, were involved in resistance emergence and maintenance (Caekebeke *et al.*, 2020; Dhaka *et al.*, 2023).

In this study, the majority of fluoroquinolone-resistant *E. coli* strains harbored QRDR mutations in *gyrA* and *parC*, regardless of the farm type, with the *gyrA* S83L/D87N double mutation and *parC* S80I mutation identified as the primary resistance mechanisms. This combination of mutations was closely associated with high-level fluoroquinolone-resistance and represented a typical pattern repeatedly reported in resistant *E. coli* isolated from domestic and international swine and livestock environments (Cheng *et al.*, 2020; Hayer *et al.*, 2020; Seo *et al.*, 2022). These findings suggested that fluoroquinolone resistance was not confined to specific farm types, but rather was based on a widely disseminated pool of resistance genes.

The PMQR gene showed similar detection rates in the integrated and conventional farms, with various types identified, including *qnrD*, *qnrS*, *qnrB*, and *aac(6')-Ib-cr*. PMQR genes confer low-level resistance alone, but significantly increase the fluoroquinolone MICs when combined with QRDR mutations, contributing to the survival and persistence of resistant strains (Seo *et al.*, 2022). In this study, the majority of strains harboring PMQR genes also carried QRDR mutations, suggesting that fluoroquinolone resistance was maintained by a multi-layered mechanism rather than by a single mechanism.

The MIC analysis revealed that the MIC_{50} and MIC_{90} values for ciprofloxacin and norfloxacin showed minimal differences between farm types. However, enrofloxacin exhibited a higher MIC distribution in the strains originating from the conventional farms. Enrofloxacin is a relatively frequently used fluoroquinolone antimicrobial in the swine industry (APQA, 2025). This difference suggested that variations in farm-level antimicrobial usage intensity and exposure history may have selectively influenced the resistance to specific fluoroquinolones. Furthermore, the observation of high MICs in some strains, despite maintaining only one *gyrA* or *parC* as a wild type, suggested the possible involvement of the PMQR gene or unidentified secondary resistance mechanisms (Wu *et al.*, 2019). This aligns with previous research findings, indicating that the expression of fluoroquinolone resistance is determined by the interaction of various genetic factors, rather than solely by the accumulation of QRDR mutations (Ruiz, 2003). In summary, the present study indicated that

fluoroquinolone-resistant *E. coli* in domestic swine environments had developed a highly sophisticated molecular resistance structure and that the farm management systems may have acted as factors regulating these subtle differences in resistance levels.

The fact that all PMQR-carrying strains exhibited ciprofloxacin resistance and frequently showed concomitant resistance to nalidixic acid and trimethoprim–sulfamethoxazole indicated that the PMQR gene acted not as a single resistance factor but rather as part of a multidrug-resistant phenotype. These results are consistent with those of previous studies, indicating that the PMQR gene, when present alongside QRDR mutations and other resistance genes, played a role in maintaining or amplifying resistance to fluoroquinolone antimicrobials (Wu *et al.*, 2019). Furthermore, the high detection frequency of *blaTEM* and *blaCTX-M* family genes in the PMQR-carrying strains, along with the identification of class I integrons and various gene cassette combinations, suggested that these strains possessed complex multidrug resistance gene platforms (Partridge *et al.*, 2018). Notably, the co-detection of gene cassettes associated with aminoglycoside and trimethoprim resistance via the integron demonstrated that selection pressure from antimicrobial use was able to simultaneously maintain and spread not only fluoroquinolone resistance but also the overall multidrug resistance framework (Partridge *et al.*, 2018; Guo *et al.*, 2021). Synthesizing these results, *E. coli* harboring PMQR genes can be interpreted not as arising solely from specific farm management systems but rather as part of a multidrug-resistant population maintained through selective pressure from diverse antimicrobials and accumulation of mobile genetic elements (Bengtsson-Palme and Larsson, 2016; Röderova *et al.*, 2017). Therefore, fluoroquinolone-resistance management strategies suggest the need for an integrated approach that considers not only the presence of the PMQR gene itself but also resistance transmission factors such as β-lactamase genes and integrons.

The MLST-based MST analysis in this study revealed that fluoroquinolone-resistant *E. coli* were not confined to specific STs but rather were distributed within genetically adjacent ST clusters, showing limited allelic variation. Notably, the co-distribution of integrated and conventional farm-derived strains within the same ST or adjacent ST nodes suggested that fluoroquinolone-resistant strains were distributed across multiple farm environments within STs showing limited allelic variation, rather than evolving independently within each farm type (Matamoros *et al.*, 2017; Hayer *et al.*, 2020; Koster *et al.*, 2023). This pattern suggested that resistant strains were distributed within STs showing limited allelic variation rather than through the rapid spread of a single high-risk clone (Matamoros *et al.*, 2017; Hayer *et al.*, 2020; Koster *et al.*, 2023). Therefore, the MST results of the present study suggested that the farm management systems may have played a role in regulating the maintenance and subtle distribution changes of resistant strains within an existing pool of resistance genes, rather than selectively amplifying specific clones.

This study included a limited number of farms and used a cross-sectional design, which restricted the generalizability of the findings and limited its ability to directly establish a causal relationship between farm management systems and fluoroquinolone resistance. Furthermore, the absence of quantitative data on actual antimicrobial use on individual farms constrained the assessment of direct associations between specific antimicrobial use and resistance levels. In addition, only fecal *E. coli* isolates were analyzed; therefore, resistant bacteria present in other farm-associated reservoirs, such as the environment or production facilities, were not evaluated. Moreover, as MLST-based analysis was utilized, the precise analysis of resistance transmission pathways at the whole-genome level requires supplementation in future research.

Conclusions: This study compared fluoroquinolone-resistant *E. coli* isolates from integrated and conventional swine farms in South Korea. Differences in antimicrobial resistance rates were observed depending on the farm type; however, QRDR mutations, PMQR gene distribution, and genetic associations were consistent across both farm types. This suggested that fluoroquinolone resistance had not newly developed in specific farm types, but rather that the resistance level and distribution were regulated based on the farm management system, utilizing a shared pool of resistance genes. The results emphasize the importance of an integrated approach for managing fluoroquinolone resistance in the swine industry, encompassing not only individual antimicrobial use restrictions, but also farm operational structures and antimicrobial management systems.

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