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Dissemination of *Escherichia coli* carrying plasmid-mediated quinolone resistance (PMQR) genes from swine farms to surroundings

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HIGHLIGHTS

- The resistance rates of *E. coli* isolates from swine farm samples against nine antibiotics were high.
- In total, 82.31% (107/130) of *E. coli* isolates carried at least one PMQR gene.
- *E. coli* carrying PMQR genes and originating from feces in swine farms could spread to the external environment.

GRAPHICAL ABSTRACT

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ABSTRACT

Different samples were collected from three swine farms in China to investigate the spread of antibiotic-resistant *Escherichia coli*. A total of 130 *E. coli* isolates were obtained from feces, air, river water, silt, and soil samples and characterized. The susceptibility of the *E. coli* isolates to 19 antibiotics was tested. The results revealed that the resistance rates of the *E. coli* isolates against 9 antibiotics were high. The minimum inhibitory concentration (MIC) values of ciprofloxacin, ofloxacin, and nalidixic acid were mainly in the ranges of 2–64, 8–64, and 8–64 μg/ml. The plasmid-mediated quinolone resistance (PMQR) genes *qnr*, *aac(6′)-Ib-cr*, *qepA*, and *oqxAB* were detected by polymerase chain reaction (PCR), and the similarity of *E. coli* from different samples was identified by pulsed-field gel electrophoresis (PFGE). The detection rates of the *qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib-cr*, *qepA*, and *oqxAB* genes in the *E. coli* isolates from three swine farms were in the range of 10.87–23.08%, 13.04–20.51%, 40.00–43.48%, 30.43–38.46%, 6.52–12.82%, and 7.69–17.39%, respectively. The PFGE result showed that 49% (49/100) of isolates originating from air, river water, soil, and silt samples had ≥85% similarity to fecal-obtained isolates, and 40.82% (20/49) of them shared the same PMQR genes with fecal-obtained isolates. This indicated that *E. coli* carrying PMQR genes and originating from feces in swine farms could spread to the external environment, which could be a potential threat to the public environment and human health.

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1. Introduction

Escherichia coli is a Gram-negative bacterium commonly found in the intestines of humans and animals (Schroeder et al., 2004; Schierack et al., 2006), and it is also the main etiologic agent of farm animal diarrhea and acute urinary tract infections (Makrina et al., 2012; Manges, 2016). It is an opportunistic pathogen that is widely present in animal farms and their surrounding environments (Duan et al., 2006; Gao et al., 2015). Quinolones are broad-spectrum antibiotics that have become the most widely used in clinical practice after several generations of development. It play an extremely important role in the treatment of bacterial infections such as *E. coli* (Michael and Jason, 2014) and are often used to treat and prevent diseases in pig farms in China (Tian et al., 2009, 2012; Yuan et al., 2009). With the use and even abuse of quinolones, especially in some developing countries, resistance to these drugs is becoming more common and spreading (Hart and Kariuki, 1998). The resistance rate against quinolones of clinical isolates of *E. coli* from human hospitals has gradually increased up to 70% in China in recent years (Zhu et al., 2011; Xiao et al., 2011).

There are four main mechanisms of *E. coli* resistance to fluoroquinolones: 1) mutations of the genes encoding the target enzyme DNA gyrase (encoded by *gyrA* and *gyrB* genes) and topoisomerase IV (encoded by *parC* and *parE*), 2) efflux of quinolones by hyperexpression of chromosomal pumps or acquisition of plasmidic pumps (e.g., *qepA*, *oqxAB*), 3) the decrease in membrane permeability, and 4) enzymatic inactivation by *AAC-(6′)-Ib-cr* (Mirzaii et al., 2018; Recacha et al., 2017). Quinolone resistance was originally thought to be mediated by chromosomal gene mutations, which block DNA replication by inhibiting DNA rotatase and topoisomerase IV. However, the plasmid-mediated quinolone resistance gene (PMQR) *qnr* was first identified in a clinical isolate of *Klebsiella pneumonia* in 1998 (Martinez-Martinez et al., 1998), followed by a second mechanism for PMQR in 2006: the modification of certain quinolones by a particular aminoglycoside acetyltransferase, *aac(6′)-Ib-cr* (Robicsek et al., 2006). A third mechanism for PMQR was added in 2007 with the discovery of plasmid-mediated quinolone efflux pumps *QepA* (Yamane et al., 2007; Périchon et al., 2007) and *OqxAB* (Hansen et al., 2007). Horizontal gene transfer of these PMQR determinants between bacteria have contributed to the decrease in susceptibility to fluoroquinolones (Gay et al., 2006; Cattoir et al., 2008; Zhou et al., 2011; Li et al., 2012), which has attracted wide attention. Studies have shown that *E. coli* isolated from different environments carries both ESBL and PMQR genes (Wang et al., 2012), and they usually coexist on the same plasmid. Plasmids carrying multiple resistance genes can transfer the multiple resistance among different strains, which poses a great threat to the treatment of human and animal diseases (Jiang et al., 2012).

Food-producing animals are considered a reservoir of resistant bacteria (Carattoli, 2008; Ma et al., 2009). Bacteria including the pathogenic ones carried by food-producing animals could spread to surrounding environments through different routes, such as air, water and waste (McEachran et al., 2015; Sanchez et al., 2016), which might result in infections in animals and farm workers (Fiegel et al., 2006; Létourneau et al., 2010; Yu et al., 2016). The direct effluence of waste to the environment and the application in agriculture have contributed to the dissemination of resistant bacteria (Buchberger, 2007; Chee et al., 2009; Liu et al., 2012). Large amounts of (fluoro)quinolone-resistant *E. coli* are contained in animal houses, especially *E. coli* carrying PMQR genes (Chen et al., 2012; Jiang et al., 2012; Wang et al., 2012). The PMQR mechanism is widely distributed in bacterial plasmids in various environments around the world (Strahilevitz et al., 2009). In the small animal hospitals in Shaanxi province, China, 80% of isolates harbored at least one PMQR gene, and 40% of isolates harbored more than one PMQR gene (*qnrB*, *qnrD*, *qnrS*, *qepA*, or *aac(6′)-Ib-cr*). At present, research on the mechanisms of fluoroquinolone resistance in *E. coli* is mainly focused on clinical isolates and pathogenic *E. coli* (Mirzaii et al., 2018; Piekarska et al., 2015). More research has been done on the resistance mechanisms of the *gyrA* and *parC* mutations, but less attention has been given to *E. coli* carrying PMQR genes isolated from farms (Piekarska et al., 2015; Gao et al., 2015). There are fewer studies on the transmission of these resistant bacteria from swine farms to the surrounding environment (Chen et al., 2012; Gao et al., 2015). Thus, in this study, *E. coli* was isolated from fecal and environmental samples from three swine farms to test the antibiotic susceptibility. The similarity of the PMQR genes was examined to investigate the spread of quinolone-resistant *E. coli* from breeding environments to the surrounding environments, which has far-reaching effects in veterinary public health and epidemiological significance.

2. Materials and methods

2.1. Swine farms

Between February and July 2014, fecal and environment samples were collected from three swine farms located in Tai'an, Shandong Province, China. The swine farms are located on the east side of the villages. The distances between the swine farms and the nearest village ranged from 500 to 800 m. All farms investigated were equipped with exhaust fans and were all naturally ventilated through the doors and windows according to the indoor temperature. The feces were removed daily and then accumulated for land application. The swine houses were disinfected once or twice per week with peracetic acid and sodium hypochlorite. The animal densities were 0.9–2.9 animals per square meter. Subtherapeutic doses of broad-spectrum antibiotics such as tylosin, penicillin, streptomycin, and tetracycline were used for prophylaxis and growth promotion.

2.2. Sampling of airborne E. coli

E. coli was collected from the air inside the swine houses using a six-stage Andersen sampler (Andersen, 1958). Samplers with MacConkey agar plates (Oxoid, CM0115) were placed in the middle of the houses approximately 80 cm above the ground, and the indoor air was collected at an airflow rate of 28.3 L/min for 1 to 5 min. Airborne *E. coli* from the outdoor air was also collected using a Reuter centrifugal sampler (RCS) at 1.5 m above the ground level with an airflow rate of 40 L/min for 0.5–8 min at two upwind sites (10 and 50 m away) and seven downwind sites (5, 10, 20, 50, 100, 200, and 400 m away). Samples were collected from each site in triplicate.

The collected samples were cultured at 37 °C for 24–48 h, and then the Gram status of the colonies was determined. Gram-negative colonies were subcultured in eosin methylene blue (EMB) agar (Tian He, Hangzhou, China) and identified by an API 20 E system (Bio Merieux, Marcy-l'Etoile, France). All isolates were stored at −20 °C in 50% glycerin.

2.3. Fecal, silt, river water, and soil sampling

Fifty fresh fecal samples were collected randomly from each swine farm, and 50 silt, river, and soil samples were collected at each different site (5, 10, 20, 50, 100, 200, and 400 m away from the outfall of the farms). All samples were collected aseptically, stored at 4 °C, and analyzed within 24 h. Fecal, silt, river, and soil samples were diluted 10-fold with 0.9% NaCl solution. Vacuum filtration (TOM-JET1 water jet

Table 1
Description of 130 *E. coli* isolates from three swine farms.

SF	F	In	Dw	R	S	So	Total
A	8	7	6	6	6	6	39
B	12	9	7	8	3	6	45
C	10	10	8	7	5	6	46

SF: swine farm; F: fecal; In: indoor air; Dw: downwind air; R: river water; S: silt; So: soil.

Table 2
Sequences of primers and annealing temperatures used for PCR.

Gene	Primers	Sequence (5′-3′)	Amplicon (bp)	Annealing (°C)	Cycling	Reference
qnrA	qnrA-F	AGAGGATTTCTCACGCCAGG	580	54	32	(Cattoir et al., 2007)
	qnrA-R	TGCCAGGCACAGATCTTGAC				
qnrB	qnrB-F	GGMATHGAAATTCGCCACTG	264	55	34	(Cattoir et al., 2007)
	qnrB-R	TTTGCYGYYCGCCAGTCGAA				
qnrS	QnrS-F	GCAAGTTCATTGAACAGGGT	428	54	32	(Cattoir et al., 2007)
	QnrS-R	TCTAAACCGTCGAGTTCGGCG				
aac(6′)-Ib-cr	aac-F	TTGCGATGCTCTATGAGTGGCTA	482	55	34	(Park et al., 2006)
	aac-R	CTCGAATGCCTGGCGTGTTT				
qepA	qepA-F	AACTGCTTGAGCCCGTAGAT	596	60	32	(Kim et al., 2009)
	qepA-R	GTCTACGCCATGGACCTCAC				
oqxAB	oqxA-F	GACAGCGTCGCACAGAATG	339	62	34	(Chen et al., 2012)
	oqxA-R	GGAGACGAGGTTGGTATGGA				
	oqxB-F	CGAAGAAAGACCTCCCTACCC	240	62	34	(Chen et al., 2012)
	oqxB-R	CGCCGCCAATGAGATACA				
qnrS1	qnrS-cs1-F	CAATCATACATATCGGCACC	642	57	36	(Wu et al., 2007)
	qnrS-cs1-R	TCAGGATAAACAACAATACCC				
qnrS2	qnrS-cs2-F	ATGGAAACCTACCGTCACACA	654	57	36	(Chen et al., 2013)
	qnrS-cs2-R	GTCAGGAAAAACAACAATACCC				
aac(6′)-Ib	aac-F	TGACCTTGCGATGCTCTATG	508	58	36	(Jiang et al., 2008)
	aac-R	TTAGGCATCACTGCGTGTTC				

air pump) was also used to concentrate the microbial populations (Reinthaler et al., 2003). The diluted samples were spread onto an EMB agar plate. After incubation at 37 °C for 18 to 20 h, colonies with a characteristic metallic sheen were selected and streaked on a MacConkey agar plate. The plate was incubated overnight at 37 °C, after which pink colonies were selected, and the isolates were assessed accordingly.

2.4. Antibiotic susceptibility test and determination of minimum inhibitory concentration (MIC) by microdilution broth method

Antibiotic susceptibility testing was carried out by a disk diffusion method on a Mueller-Hinton agar plate with *E. coli* ATCC 25922 as a reference strain. The susceptibility of the following antibiotics was tested according to the manufacturer's instructions: ampicillin 10 μg (AMP), piperacillin 100 μg (PIP), amoxicillin 20 μg/clavulanic acid 10 μg (AMC), ampicillin 10 μg/sulbactam 10 μg (A/S), piperacillin 100 μg/sulbactam 10 μg (P/T), cephalothin 30 μg (CEP), cefuroxime 30 μg (CXM), aztreonam 30 μg (ATM), gentamicin 10 μg (GEN), kanamycin 30 μg (KAN), streptomycin 10 μg (STM), amikacin 30 μg (AMK), tetracycline 30 μg (TET), ciprofloxacin 5 μg (CIP), ofloxacin 5 μg (OFX), nalidixic acid 30 μg (NAL), sulfamethoxazole 25 μg (SXT), chloramphenicol 30 μg (CAP), and nitrofurantoin 300 μg (NIT) (Tan Hou Ocean Development Co., Ltd., Hangzhou, China).

Ciprofloxacin, ofloxacin, and nalidixic acid were diluted to different concentrations, and then the MIC values of tested bacteria and reference strain (ATCC25922) were detected. The test method, judgment standard, and quality control test were carried out by the American Clinical Laboratory Standardization Committee (NCCLS, 2004).

2.5. Identification of the plasmid-mediated quinolone resistance genes

The DNA templates of the *E. coli* isolates were prepared with a boiling method. PCR was used to detect the antibiotic-resistance genes *qnrA*, *qnrB*, *qnrS*, *qnrS1*, *qnrS2*, *aac (6′)-Ib*, *aac (6′)-Ib-cr*, *qepA*, and *oqxAB*. The primer sequences and annealing temperatures are listed in Table 2. The amplification reactions were performed as previously described (Cheng et al., 2006). Each sample was tested three times, and isolates that were confirmed by sequence and blast analysis were used as quality controls.

Table 3
Occurrence of antimicrobial resistance among *E. coli* isolates obtained from different samples.

Drug	Swine farm A							Swine farm B							Swine farm C						
	F n = 8	In n = 7	Dw n = 6	R n = 6	So n = 6	S n = 6	Total n = 39	F n = 12	In n = 10	Dw n = 7	R n = 8	So n = 6	S n = 3	Total n = 45	F n = 10	In n = 10	Dw n = 8	R n = 7	So n = 6	S n = 5	Total n = 46
AMP	4	5	4	3	4	5	25	9	7	5	3	3	2	29	8	10	8	5	5	3	39
PIP	4	4	4	3	2	5	22	3	2	3	1	3	1	13	3	6	6	3	2	2	22
AMC																				1	1
A/S																					
P/T																					
CEP	1	1		2		4	8	1	2	3	1	3	2	12	7	7	4	4	3	3	28
CXM	2	2					4	2	2	2	1	1	2	10	6	7	4	4	3	3	27
ATM								2	1	1			2	6							
GEN	5	3	4	5	6	6	29	9	8	5	6	4	1	33	7	6	7	6	5	5	36
KAN	6	5	4	5	6	5	31	11	7	5	4	2	1	30	8	7	5	3	5	3	31
STM	4	5	5	5	6	5	30	9	8	6	5	3	1	32	5	8	6	7	5	4	35
AMK													1	1							
TET	6	6	6	6	6	6	36	12	9	5	8	5	3	42	10	9	7	7	6	5	44
CIP	8	5	6	2	5	4	30	8	7	3	6	4	2	30	8	7	7	6	4	4	36
OFX	7	4	5	5	4	4	29	9	7	4	4	3	1	28	9	8	7	6	3	2	35
NAL	8	7	5	4	4	3	31	9	6	5	5	6	1	32	9	9	6	5	4	5	38
SXT	6	5	6	3	4	2	25	8	6	3	5	3	2	27	8	8	6	6	4	3	35
CAP	4	2		3	1		10	3	2	1	1		1	8	3	1					4
NIT																				1	1

F: fecal; In: indoor air; Dw: downwind air; R: river; So: soil; S: silt.

Table 4
E. coli carrying PMQR genes from different sources.

		0 PMQR gene	1 PMQR gene	2 PMQR genes	3 PMQR genes	4 PMQR genes
Swine farm A	F	0	2	5	1	0
	In	0	5	1	1	0
	Dw	0	2	4	0	0
	R	1	2	2	1	0
	S	0	5	1	0	0
	So	3	2	1	0	0
Swine farm B	F	1	6	3	2	0
	In	3	3	3	0	0
	Dw	0	5	1	0	1
	R	3	4	1	0	0
	S	1	1	0	1	0
	So	1	5	0	0	0
Swine farm C	F	0	3	5	2	0
	In	4	5	0	1	0
	Dw	2	4	2	0	0
	R	0	7	0	0	0
	S	2	1	2	0	0
	So	2	1	1	1	1
Total		23	63	32	10	2

2.6. Qnr gene sequence determination and analysis

The 58 *qnrS* PCR positive product and 21 *qnrB* PCR positive product were purified and ligated into the PMD18-T cloning vector and then transferred into DH5α competent cells. The positive strain that had been successfully cloned with the *qnrS* target fragment was cultured overnight and sent for sequencing to the Tsingke company (Beijing, China). Its gene subtype was determined according to the database of *qnr* allele designations at http://www.lahey.org/qnrStudies.

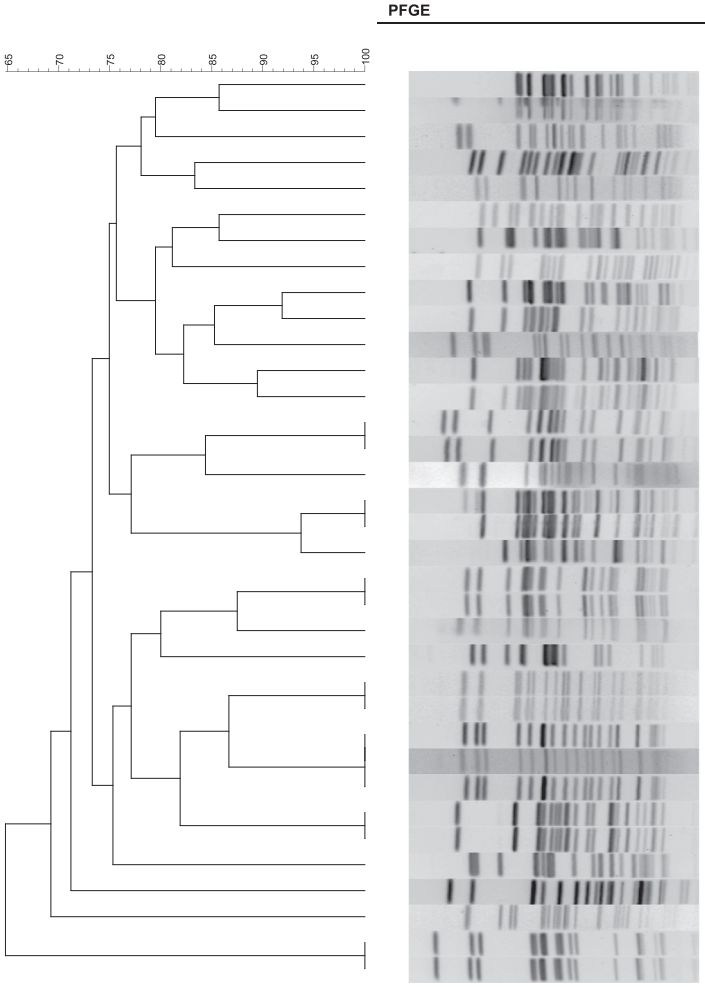
2.7. Pulsed field gel electrophoresis (PFGE)

To determine the genetic relatedness of the *E. coli* isolates, their genomic DNA were digested with the restriction enzyme *Xba*I and then subjected to PFGE analysis according to the PulseNet Standardized Laboratory Protocol (PulseNet., 2002) using the CHEF Mapper™ System (Bio-Rad Laboratories, Hercules, CA) and previous literature (Tenover et al., 1995). The *Salmonella* (H9812) serotype Braenderup strain restricted with *Xba*I was used as a molecular weight standard. Cluster analysis of the pulsotypes was carried out using the Dice coefficient method in UPGMA (optimization: 1.5%; band position tolerance: 1.5%) in BioNumerics (Version7.6, Applied Maths, Inc.).

3. Results

3.1. Isolation of E. coli

In total, 130 *E. coli* isolates were obtained from the three swine farms, including 30 fecal isolates, 26 indoor air isolates, 21 downwind air isolates, 21 river water isolates, 14 silt isolates, and 18 soil isolates (Table 1). No isolates were obtained from upwind air samples.



Strains	Sources	PFGE Subtypes	PMQR Genes
Silt10m-3	Silt	Y-SFA	*aac(6 ')-Ib-cr*
River100m-1	River	X-SFA	*qnrB31, qnrS2*
Silt10m-2	Silt	H"-SFA	*qnrS1*
Dw400m-1	Downwind	M"-SFA	*aac(6 ')-Ib-cr ,qepA*
Fecal-8	Fecal	L"-SFA	*qnrB2,aac(6 ')-Ib-cr ,oqxAB*
Dw100m-2	Downwind	H-SFA	*qnrA,qnrS1*
Indoor-3	Indoor air	J-SFA	*qnrA,qnrS1*
Dw10m-1	Downwind	K-SFA	*qnrS1*
Fecal-3	Fecal	L-SFA	*aac(6 ')-Ib-cr ,qepA qnrS1,*
Fecal-4	Fecal	N-SFA	*aac(6 ')-Ib-cr*
Silt20m-1	Silt	I"-SFA	*aac(6 ')-Ib-cr,qepA*
Silt50m-1	Silt	O-SFA	*qnrA*
Fecal-6	Fecal	A"-SFA	*qnrA*
River10m-1	River	K"-SFA	*qnrB31, qnrS1*
River50m-1	River	K"-SFA	*aac(6 ')-Ib-cr*
River200m-2	River	T-SFA	*qnrB9, aac(6 ')-Ib-cr ,oqxAB*
Indoor-4	Indoor air	B"-SFA	*qnrS1*
Silt5m-1	Silt	B"-SFA	*qnrA*
River200m-1	River	C"-SFA	*qnrS2*
Soil200m-1	Soil	C-SFA	*qnrS2*
Fecal-2	Fecal	C-SFA	*qnrS2, aac(6 ')-Ib-cr*
Indoor-1	Indoor air	E-SFA	*qnrA*
Dw200m-1	Downwind	G-SFA	*qnrS1, aac(6 ')-Ib-cr*
Soil400m-1	Soil	U-SFA	*qnrB2, aac(6 ')-Ib-cr*
Indoor-2	Indoor air	U-SFA	*qnrB2, aac(6 ')-Ib-cr ,oqxAB*
Fecal-7	Fecal	V-SFA	*qnrS1*
Indoor-7	Indoor air	V-SFA	*qnrB9*
Fecal-5	Fecal	V-SFA	*qnrA, qnrS1*
Dw100m-1	Downwind	W-SFA	*qnrS1, aac(6 ')-Ib-cr*
Indoor-6	Indoor air	W-SFA	*qepA*
Soil400m-2	Soil	S-SFA	*aac(6 ')-Ib-cr*
Indoor-5	Indoor air	N"-SFA	*qnrS2*
Dw50m-1	Downwind	O"-SFA	*qnrA*
Silt5m-2	Silt	P"-SFA	*qnrA*
Fecal-1	Fecal	P"-SFA	*aac(6 ')-Ib-cr ,qepA*

Fig. 1. PFGE dendrogram of *E. coli* isolates from swine farm A (SFA) and the PMQR genes tested.

3.2. Antibiotic susceptibility test and determination of MIC value of ciprofloxacin, ofloxacin, and nalidixic acid

The *E. coli* strains showed diverse susceptibility among swine farms (Table 3). On the whole, the resistance rates were over 90% against TET, 64.10–84.78% against AMP, 66.67–79.49% against KAN, 60.00–76.09% against SXT, and over 70% against GEN and STM. The isolates resistant to CIP from fecal, indoor air, downwind air, river, soil and silt samples were #24, #19, #16, #14, #12, and #10, respectively; those resistant to OFX were #25, #19, #16, #15, #10, and #7; and those resistant to NAL were #26, #22, #16, #14, and #9. Isolates from all three farms demonstrated susceptibility to A/S, P/T, ATM, and AMK. In addition, six isolates from farm B were resistant to ATM.

All *E. coli* isolates from the three farms were resistant to at least three antibiotics. Resistance to eight antibiotics (23.08%) was the most prevalent phenotype among all strains, followed by resistance to seven antibiotics (15.38%). *E. coli* isolates obtained from farm A were resistant to 3–11 antibiotics, while isolates from farms B and C were resistant to 3–13 antibiotics.

The MICs of the 130 *E. coli* isolates were determined by the microdilution method. The MIC values of ciprofloxacin, ofloxacin, and nalidixic acid were mainly in the ranges of 2–64, 8–64, and 8–64 μg/mL, which correspond to low-medium resistance and less highly resistant.

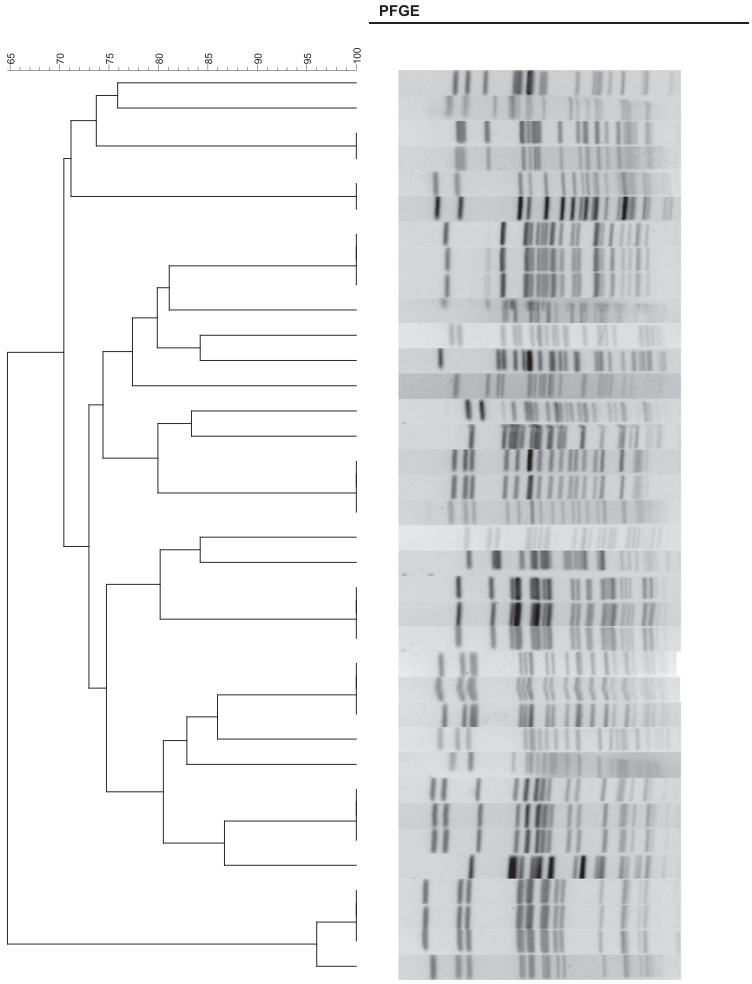
3.3. Detection results of the plasmid-mediated quinolone resistance genes

The genes detected among the 130 *E. coli* isolates were *qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib*, *aac(6′)-Ib-cr*, *qepA*, and *oqxAB*. In total, 82.31% (107/130) of the *E. coli* isolates carried at least one PMQR gene, and Table 4 shows the different association patterns of PMQR genes per strain (including for each type of sample: fecal, indoor air, downwind air, and so on). The results showed that 63 isolates carried one PMQR gene, 32 isolates carried two PMQR genes, 10 isolates carried three PMQR genes, and 2 isolates carried four PMQR genes. The detection rates of the *qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib*, *aac(6′)-Ib-cr*, *qepA*, and *oqxAB* genes in the *E. coli* isolates from the three swine farms were in the ranges of 10.87–23.08%, 13.04–20.51%, 40.00–43.48%, 31.11–41.03%, 30.43–38.46%, 6.52–12.82%, and 7.69–17.39%, respectively.

All *oqxA*-positive isolates were also screened for *oqxB*. The dominant PMQR gene was the *qnrS* gene, and the detection rate of the *qepA* gene was the lowest among all the swine farms investigated. The *QepA* gene was detected in five, four, and three isolates in the three farms, respectively.

3.4. Sequence analysis of bacteria resistance genes qnrS and qnrB

The results of the 58 PCR positive product sequencing was 54 strains of *qnrS* and 4 false-positive results. In addition, the typing results of the 54 *qnrS* positive strains showed that 39 of them belonged to *qnrS1* and 15 belonged to *qnrS2*. The result of 21 *qnrB* PCR positive product sequencing was 19 strains of *qnrB* and 2 false-positive results. The typing results of 19 *qnrB* positive strains showed that 10 of them belonged to *qnrB2*, six belonged to *qnrB9*, and three belonged to *qnrB31*.



Strains	Sources	PFGE subtypes	PMQR Genes
Fecal-10	Fecal	A-SFB	*qnrA, qnrS2*
Dw200m-1	Downwind	E-SFB	*aac(6 ')-Ib-cr*
Soil100m-1	Soil	S-SFB	*qnrS1*
River100m-1	River	S-SFB	*qnrS2*
Soil50m-1	Soil	N"-SFB	*qnrS2*
Fecal-2	Fecal	N"-SFB	*aac(6 ')-Ib-cr ,qepA, oqxAB*
River10m-2	River	W-SFB	*qnrS1*
Dw10m-1	Downwind	W-SFB	*qnrB2*
Dw100m-1	Downwind	W-SFB	*qnrB2*
River200m-2	River	X-SFB	*qepA, aac(6 ')-Ib-cr*
Soil200m2	Soil	H"-SFB	*qnrB2*
Dw50m-2	Downwind	Z-SFB	*qnrS1*
Indoor-9	Indoor air	O"-SFB	*qnrS2*
Soil200m-3	Soil	F"-SFB	*qnrS1*
Indoor3	Indoor air	B"-SFB	*qepA*
Fecal-1	Fecal	V-SFB	*aac(6 ')-Ib-cr*
Fecal-9	Fecal	V-SFB	*qnrA,qnrS1*
Fecal-4	Fecal	V-SFB	*qnrA, aac(6 ')-Ib-cr ,oqxAB*
Fecal-11	Fecal	K-SFB	*qnrS1, aac(6 ')-Ib-cr*
River50m-1	River	J-SFB	*qepA*
Fecal-8	Fecal	L-SFB	*qnrS1*
Indoor-1	Indoor air	L-SFB	*qnrS1*
Fecal-6	Fecal	L-SFB	*qnrS1*
Dw200m-2	Downwind	I"-SFB	*qnrA, qnrB9, aac(6 ')-Ib-cr, oqxAB*
Indoor-5	Indoor air	I"-SFB	*aac(6 ')-Ib-cr ,oqxAB*
Indoor-6	Indoor air	I"-SFB	*qnrA, qnrS1*
Fecal-7	Fecal	J"-SFB	*aac(6 ')-Ib-cr*
Soil400m-1	Soil	T-SFB	*aac(6 ')-Ib-cr*
Indoor-4	Indoor air	K"-SFB	*qnrS1, aac(6 ')-Ib-cr*
Fecal-3	Fecal	K"-SFB	*qnrB2*
River50m-2	River	K"-SFB	*qnrS1*
Dw10m-2	Downwind	D"-SFB	*qnrS2*
Dw50m-1	Downwind	P"-SFB	*aac(6 ')-Ib-cr ,qepA*
Fecal-12	Fecal	P"-SFB	*aac(6 ')-Ib-cr*
Silt50m-1	Silt	P"-SFB	*qnrB9,aac(6 ')-Ib-cr ,oqxAB*
Silt10m-1	Silt	Q"-SFB	*qnrS2*

Fig. 2. PFGE dendrogram of *E. coli* isolates from in swine farm B (SFB) and the PMQR genes tested.

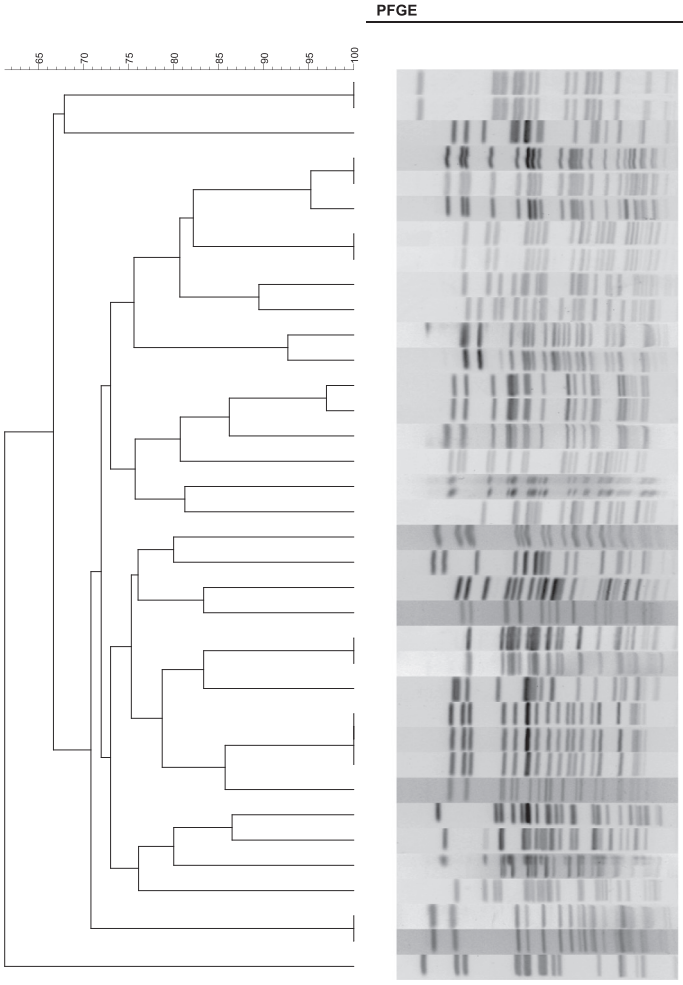
3.5. PFGE

The *E. coli* isolates showed diverse PFGE profiles, which fully reflected the genetic polymorphism of *E. coli*. The similarity of the *E. coli* isolates was 65–100% in the three swine farms. The PFGE results showed >85% similarity in 14, 11, 9, 7, and 10 isolates from indoor air, outdoor air, soil, silt, and river samples, respectively. In swine farm A (Fig. 1), the samples Dw100m-2 and Indoor-3 had >85% similarity to the Fecal-5 sample and carried the same PMQR genes: *qnrA* and *qnrS*. In swine farm B (Fig. 2), the samples Dw10m-1, Dw100m-1, and Soil 200m-2 had >85% similarity to the sample Fecal-3 and carried the same PMQR gene, *qnrB*. The similarity between the samples Fecal-8 and Indoor-1 was 100%, and both carried the *qnrS* genes. In swine farm C (Fig. 3), the samples River100m-2, Indoor-6, and Soil100m-2 had above 85% similarity to Fecal-1 and carried the same PMQR gene, *qnrS*. The similarity between samples Fecal-3 and Soil200m-3 was 100%, and both carried *qnrS* genes and *aac(6′)-Ib-cr* genes. Some isolates were detected in multiple types of samples along the environmental transmission gradient from feces to soil, and they carried the same resistance genes.

4. Discussion

In this study, 130 *E. coli* Isolates from different swine farms all showed high resistance against (fluoro) quinolones (CIP, OFX, and NAL), aminoglycosides (GEN, KAN, and STM), TET, SXT, and AMP. The resistance rate against (fluoro)quinolones was in the range of 74.36–82.61%, which is consistent with previous studies (Gao et al., 2015; Zhu et al., 2011; Xiao et al., 2011). Furthermore, 93.08% (121/130) of the isolates were sensitive to at least 5 antibiotics, and some were sensitive to as many as 10–13 antibiotics. Antibiotic susceptibility was similar between farms, but there was also a large difference in drug resistance among individuals, which may be related to the different management systems of drug use in the different farms. This indicated that the high resistance rate might be related to the multiple and inappropriate use of these antibiotics (Teuber, 2001; Lipsitch et al., 2002; Rosa and Carlos, 2013). The PMQR genes were very common in the farms, with 82.31% (107/130) of the *E. coli* isolates carrying at least one. *qnrS* and *aac(6′)-Ib-cr* were the main PMQR genes, and multiple PMQR genes coexisted in the same isolates, which improves the resistance of the bacteria to antimicrobial drugs. In the *qnr* gene family, two variants of *qnrS* were detected: *qnrS1* and *qnrS2*. There are many variants of *qnrB*, the most easily mutated genotype (Wang et al., 2011), which is consistent with our results. Variable qnr resistance genes in *E. coli* could significantly improve the resistance to quinolones.

In animal houses, there are large amounts of feces and waste containing resistant bacteria, which could enter into the air through animal activities and form into bio-aerosols. The transmission of bio-aerosols carrying pathogens or resistant bacteria could pose threats for animals and public health. PFGE experiments on *E. coli* with different sources in different swine house showed that their similarity was very high (as much as 100%). A total of 47 *E. coli* isolates were obtained from indoor air samples and outdoor air samples at 10 m, 50 m, 100 m, 200 m, and 400 m downwind. PFGE showed that 25 isolates were clonally related to fecal isolates, including 9 isolates carrying the same PMQR genes, which indicates that airborne transmission was a possible route



Strains	Sources	PFGE Subtypes	PMQR Genes
Fecal-7	Fecal	B-SFC	*qnrS1, oqxAB*
Indoor-3	Indoor air	B-SFC	*qnrS1*
Indoor-9	Indoor air	A-SFC	*qnrS1*
River200m-2	River	Q-SFC	*qnrB2*
Indoor-4	Indoor air	Q-SFC	*qnrA*
Soil100m-2	Soil	R-SFC	*qnrS2*
Fecal-4	Fecal	K-SFC	*aac(6 ')-Ib-cr ,qepA, oqxAB*
Dw10m-1	Downwind	K-SFC	*aac(6 ')-Ib-cr*
River100m-2	River	I-SFC	*qnrS2*
Fecal-1	Fecal	H-SFC	*qnrS2*
Indoor-5	Indoor air	G"-SFC	*aac(6 ')-Ib-cr ,qepA, oqxAB*
Silt5m-1	Silt	F"-SFC	*qnrS1, aac(6 ')-Ib-cr*
Fecal-6	Fecal	D-SFC	*qnrB2, qnrS1*
Dw10m-2	Downwind	C-SFC	*aac(6 ')-Ib-cr*
Fecal-10	Fecal	E-SFC	*qnrS1, aac(6 ')-Ib-cr, oqxAB*
Fecal-9	Fecal	F-SFC	*qnrA, aac(6 ')-Ib-cr*
River200m-1	River	M-SFC	*qnrS1*
River10m-1	River	P-SFC	*qnrB9*
Dw50m-1	Downwind	I"-SFC	*aac(6 ')-Ib-cr*
Fecal-8	Fecal	K"-SFC	*qnrS1*
River50m-1	River	M"-SFC	*qnrB2*
Fecal-5	Fecal	L"-SFC	*qnrB9, qnrS1*
Soil100m-1	Soil	B"-SFC	*qnrA, aac(6 ')-Ib-cr , qepA, oqxAB*
Dw400m-1	Downwind	B"-SFC	*qnrB31, qnrS1*
Silt20m-2	Silt	E"-SFC	*qnrS1*
Indoor-6	Indoor air	V-SFC	*qnrS2*
Indoor-10	Indoor air	V-SFC	*aac(6 ')-Ib-cr*
River100m-1	River	V-SFC	*qnrA*
River10m-2	River	U-SFC	*qnrS1*
Dw50m-2	Downwind	Z-SFC	*aac(6 ')-Ib-cr ,oqxAB*
Soil200m-1	Soil	W-SFC	*qnrA, aac(6 ')-Ib-cr ,oqxAB*
Silt50m-1	Silt	X-SFC	*aac(6 ')-Ib-cr ,oqxAB*
Fecal-2	Fecal	O"-SFC	*qnrS1*
Soil200m-3	Soil	N"-SFC	*qnrS1,aac(6 ')-Ib-cr*
Fecal-3	Fecal	N"-SFC	*qnrS1, aac(6 ')-Ib-cr*
Dw50m-3	Downwind	P"-SFC	*qnrS1*

Fig. 3. PFGE dendrogram of *E. coli* isolates from swine farm C (SFC) and the PMQR genes tested.

for *E. coli* carrying PMQR genes. In addition, the same origin as fecal isolates was observed in 10 and 9 *E. coli* isolates from river water at 10 m, 50 m, 100 m, and 200 m away and from soil at 50 m, 100 m, 200 m, and 400 m away. Furthermore, 7 *E. coli* isolates from silt at 5 m, 10 m, and 20 m were clonally related to fecal isolates. This indicates that *E. coli* carrying PMQR genes and originating from indoor feces could transmit between the internal and external environments of the swine farms through the river, air, soil, and silt.

It has been reported that approximately 75% of antibiotics cannot be absorbed by animals and leak into the waste (Donohoe, 1984; Chee et al., 2009). Although some antibiotics are discharged into surrounding rivers during irrigation or fertilization processes, most antibiotics in the open-air lagoons are re-applied to the surrounding agricultural fields. Significant amounts of antibiotics can be found in manure and waste slurries (Gavalchin and Katz, 2015; Pedro et al., 2009). Thus, antibiotic-resistant bacteria from indoor feces could spread to surrounding environments and pollute the air (≥400 m), river (≥200 m), soil (≥400 m), and silt (≥200 m).

The transmission distance varies with the media, which might be due to the high mobility of air and river water and the low mobility of silt. The distance was much longer in soil than in river water, which might be related to the artificial transmission by villagers' farming activities like irrigation, and this artificial spread could be unlimited (McEachran et al., 2015; Sanchez et al., 2016; Liu et al., 2012). The transmission of antibiotic-resistant *E. coli* not only poses a threat of infection to the animals raised but also tends to cause the spread of epidemic diseases. The detailed influences and mechanisms behind this transmission should be investigated further.

5. Conclusion

The present study revealed that *E. coli* carrying PMQR genes and originating from feces in swine farms could spread to the external environment. These bacteria pollute the nearby air, water, silt, and soil, which could be a potential threat to the public environment and human health.

Conflict of interest statement

The authors have no conflicts of interest to declare.

CRediT authorship contribution statement

Bo Wu: Conceptualization, Data curation, Writing - original draft, Writing - review & editing. **Qin Qi:** Data curation. **Xiaodan Zhang:** Data curation. **Yumei Cai:** Formal analysis. **Guanliu Yu:** Formal analysis. **Jing Lv:** Formal analysis. **Lili Gao:** Writing - review & editing. **Liangmeng Wei:** Writing - review & editing. **Tongjie Chai:** Conceptualization, Funding acquisition, Writing - original draft.

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