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Research **OPEN ACCESS**

Detection of quinolone antibiotic resistance genes in *Escherichia coli* isolated from dairy cattle feces

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Abstract

Background Livestock raised in densely populated areas can serve as reservoirs for bacteria such as *Escherichia coli*, which may harbor antibiotic resistance genes that threaten both animal and human health.

Objective This study aimed to identify and characterize quinolone resistance genes in *E. coli* isolated from dairy cattle feces.

Methods Fifteen *E. coli* isolates were obtained from 15 dairy farms located in Kebon Pedes, Bogor, West Java. Genotypic detection of quinolone resistance genes was conducted using DNA sequencing on the MinION platform.

Results All *E. coli* isolates (100%) carried at least one quinolone resistance gene. Of these, ten isolates (67%) contained a single resistance gene, while five isolates (33%) possessed two genes. The *qnrS1_1* gene was identified in all isolates and represented the predominant genotype, whereas the *qnrVC4_1* gene was found in five isolates (33%), mostly co-occurring with *qnrS1_1*. Both genes are plasmid-mediated and categorized as plasmid-mediated quinolone resistance (PMQR) genes.

Conclusion The detection of *qnrS1_1* and *qnrVC4_1* genes in *E. coli* isolated from dairy cattle feces indicates that livestock manure may act as a reservoir for quinolone resistance genes, contributing to their persistence and potential spread within farm environments.

Keywords antibiotic resistance genes | dairy cattle | DNA sequencing | *Escherichia coli* | quinolone resistance

Introduction

Residential areas located near livestock farms create an environment where farm animals may act as potential reservoirs for bacteria, particularly *Escherichia coli* (Elsharkawy *et al.*, 2024). According to Loncaric *et al.* (2013), *E. coli* can be detected in various environmental matrices such as soil, water, air, and dust, as a result of contamination from animal feces. The bacterium may also be present on equipment used in routine farming activities, reflecting its ability to persist and to be transmitted within agricultural environments.

Antibiotics are commonly administered to livestock to treat bacterial infections. In regions with a high risk of disease transmission, antibiotic usage tends to be more frequent, not only for therapeutic purposes but also as a preventive measure. Globally, antimicrobial consumption in the food production sector has reached an alarming level, highlighting the continued overuse of antibiotics in animal agriculture, often for growth promotion and prophylaxis rather than for

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treatment. Under the business-as-usual scenario, antibiotic use in livestock is projected to reach 143,481 tons by 2040, representing a 29.5% increase from the 2019 baseline of 110,777 tons. This projected rise parallels the expansion of livestock production to meet the increasing global demand for animal-based food products (Acosta *et al.*, 2025). Such escalation raises serious concerns regarding the emergence and dissemination of antimicrobial resistance (AMR), driven by both genetic mutations and the horizontal transfer of resistance genes among bacteria via plasmids (Davies & Davies, 2010).

Among the antibiotic classes associated with AMR, quinolones are of particular concern. Quinolones are of major clinical importance because of their broad-spectrum antibacterial activity and distinct mechanism of action, which targets bacterial DNA gyrase and topoisomerase IV (Millanao *et al.*, 2021). The extensive and often overlapping use of antibiotics in human and veterinary medicine underscores their pivotal role within the One Health framework, which recognizes the interconnectedness of humans, animals, and the environment in the emergence and transmission of antimicrobial resistance (Pandey *et al.*, 2024). Quinolones are widely employed in the treatment of respiratory and urinary tract infections, as well as certain gastrointestinal diseases caused by both Gram-positive and Gram-negative bacteria (Millanao *et al.*, 2021). A country-level analysis demonstrated that the consumption of quinolones in food-producing animals is significantly and positively correlated with the prevalence of fluoroquinolone resistance in major Gram-negative pathogens such as *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Kenyon, 2021).

High levels of ciprofloxacin-resistant *E. coli* have also been documented in chickens, humans, and the surrounding environment, suggesting a shared reservoir of resistance across sectors (Das *et al.*, 2023). In dairy cattle, quinolone antibiotics are frequently administred to treat mastitis, both during lactation and the dry period, and sometimes for prophylactic purposes. Investigating quinolone resistance genes is particularly important because these antibiotics act directly on bacterial DNA, differing from other antimicrobial classes such as β-lactams that inhibit cell wall synthesis (Sharma *et al.*, 2009). Repeated and imprudent use, especially prophylactic administration, can accelerate the proliferation of antibiotic-resistant bacterial populations (Gruet *et al.*, 2001).

A study by Wichmann *et al.* (2014) revealed that dairy cattle manure contains at least 80 antibiotic resistance genes from multiple antimicrobial classes, including β-lactams, aminoglycosides, tetracyclines, quinolones, and phenicols. Similarly, Xie *et al.* (2016) identified 121 resistance genes in manure samples from dairy farms, reinforcing the notion that livestock waste serves as a significant environmental reservoir for antimicrobial resistance genes (ARGs). The application of processed dairy cattle manure as fertilizer can further facilitate the dissemination of resistance genes into the soil, sometimes within only a few days after application (Udikovic-Kolic *et al.*, 2014). Poor livestock waste management practices exacerbate this problem by accelerating the spread of resistance genes into the broader environment (Bürgmann *et al.*, 2018). Therefore, this study aimed to detect and analyze quinolone resistance genes in *E. coli* isolates obtained from dairy cattle fecal samples by genotyping using the sequencing technology provided by Oxford Nanopore Technologies (ONT).

Methods

Study period and location

This study was conducted between December 2024 and February 2025. Deoxyribonucleic acid (DNA) extraction and detection of quinolone antibiotic resistance genes (ARGs) using DNA sequencing procedures were performed at the Biotechnology Laboratory, Center for Quality Testing and Certification of Animal Products (BPMSPH), Bogor City, West Java, Indonesia.

Samples and bacterial isolates

The *E. coli* isolates used in this study were confirmed in a previous investigation (Elsharkawy *et al.*, 2024). A total of 15 isolates were obtained from pooled dairy cattle fecal samples collected from 15 farms in the Kebon Pedes Dairy Farming Area, Bogor City. Approximately 1 g of each fecal sample was suspended in sterile phosphate-buffered saline (PBS), serially diluted, and streaked on MacConkey agar for *E. coli* isolation. Colonies with characteristic morphology were purified on nutrient agar, confirmed to be *E. coli*, and indexed prior to DNA extraction. The extracted DNA was subsequently subjected to genotypic molecular analysis based on DNA sequencing using Oxford Nanopore Technologies (ONT).

Each identified *E. coli* isolate was cultured on nutrient agar (NA) and harvested using a sterile 1 µL inoculation loop into a microtube containing 1 mL phosphate-buffered saline (PBS). The suspension was centrifuged at 12,000 × g for 5 min to obtain a bacterial pellet at the bottom of the tube. The supernatant was carefully removed using a micropipette to avoid loss of the pellet, which was then used for DNA extraction.

Bacterial DNA extraction

Genomic DNA was extracted using the DNeasy® PowerWater® Kit (Qiagen, Germany) following the manufacturer's protocol, with minor modifications to optimize yield from cultured isolates. The bead-beating step was retained to ensure efficient cell lysis, and incubation times were adjusted to maximize DNA recovery. The PW1 solution was pre-incubated at 55°C for 5–10 min before use. The *E. coli* pellet was resuspended in 1 mL PW1 by pipetting up and down, transferred into a PowerWater Bead Pro Tube containing beads, homogenized using a tube shaker at maximum speed for 5 min, and centrifuged at 2,500 rpm for 1 min. The supernatant was carefully transferred to a clean 2 mL microtube and centrifuged at 13,000 × g for 1 min. The resulting DNA-containing supernatant was transferred into a new 2 mL microtube.

Two hundred microliters of IRS solution were added, mixed by pipetting, and incubated at 2–8°C for 5 minutes, followed by centrifugation at 13,000 × g for 1 minute. The

supernatant was transferred into a clean 2 mL microtube, mixed with 650 µL of PW3 solution, and applied to an MB spin column. After centrifugation at 13,000 × g for 1 minute, the flow-through was discarded. This step was repeated until all DNA–PW3 mixture had passed through the filter. The spin column was washed sequentially with 650 µL PW4 and 650 µL ethanol, and centrifuged at 13,000 × g for 1 min after each step, discarding the flow-through each time.

The column was centrifuged at 13,000 × g for 2 min to remove residual ethanol and then transferred into a new 2 mL Eppendorf DNA LoBind tube. The DNA was eluted by adding 100 µL of EB solution, centrifuging at 13,000 × g for 1 min, and discarding the column. The resulting DNA eluate was stored for subsequent analyses.

DNA quality control and sequencing

Prior to sequencing, extracted DNA was subjected to quality control (QC) assessment using a Qubit™ 4 Fluorometer (Thermo Fisher Scientific, USA), with a target concentration above 50 ng/µL, as recommended by the manufacturer. DNA concentration measurements determined the optimal amount of template required for sequencing; the higher the concentration, the less template was required. Qubit™ assay buffer was prepared according to the number of isolates, consisting of the dye and standard solution mixed thoroughly. DNA quality was measured by mixing the DNA extract with the assay buffer at a 1:199 ratio (µL).

DNA sequencing was performed using the ONT Rapid Barcoding Kit (SQK-RBK110.96), which is a non-PCR-based library preparation kit that does not require specific primers. Sequencing adapters and barcodes were directly ligated to the genomic DNA according to the manufacturer's protocol.. DNA samples (2–4 µL, corresponding to approximately 50 ng of total DNA) were diluted with nuclease-free water (NFW) to a final volume of 9 µL. One microliter of Rapid Barcode (RB01–96) was added to each tube, mixed by pipetting, and briefly spun. The tubes were incubated at 30°C for 2 min, followed by incubation at 80°C for 2 min in a thermomixer, and then cooled on ice.

All barcoded samples were pooled in a single Eppendorf DNA LoBind tube and mixed with an equal volume of AMPure XP beads (1:1). After 5 min of incubation at room temperature, the tubes were placed on a magnetic rack. The beads were washed twice with 1.5 mL of 80% ethanol, and the supernatant was discarded each time. After drying, the beads were resuspended in 15 µL of EB buffer, incubated for 10 min, placed on a magnetic rack for 1 min, and the eluate was transferred into a clean tube. DNA quality was checked again using a Qubit™ 4 fluorometer.

For sequencing, 11 µL of DNA was mixed with 1 µL Rapid Adapter F (RAP F), incubated for 5 min at room temperature, and then combined with sequencing buffer II (SBII), loading beads II (LBII), flush tether (FLT), and flush buffer (FB) as per the priming protocol. Flow cell priming was performed by replacing 200 µL of the original buffer with 800 µL of freshly prepared FLT–FB mixture, followed by a 5-minute wait. The prepared sequencing mix (37.5 µL SBII, 25.5 µL LBII, and 12 µL DNA) was loaded into the SpotON port in 75 µL increments. The MinION device was then assembled and sequencing was initiated.

Data analysis

Raw sequencing data in the FASTQ format were generated using the Oxford Nanopore EPI2ME platform. Data processing was performed on a Linux-based system in the following order: concatenation of files, filtering with Filtlong, quality assessment with NanoStat, assembly using Flye, and polishing with Medaka, followed by homogenization. The final assembly quality was assessed using QUAST and CheckM. Detection of ARGs was performed using ResFinder version 0.4.0, and plasmid sequences were identified using PlasmidFinder version 2.1.6+ galaxy1, both available on the Galaxy Europe platform. ResFinder was used to identify resistance genes from sequencing data, whereas PlasmidFinder detected plasmid sequences from whole-genome sequencing (WGS) assemblies. The resulting data were compiled and summarized using Microsoft Excel for data visualization and presentation.

Results

Sequencing data quality

Quality assessment of the sequencing data obtained from *E. coli* isolates collected from dairy cattle feces in Kebon Pedes, Bogor City, demonstrated that the generated assemblies were of high quality. The average genome length was 207,266,692 ± 10,997,926 bp, with completeness values exceeding 90% and contamination levels below 5%. The mean N50 value reached 4,450 ± 339 bp (**Table 1**). These quality metrics collectively indicate that the sequencing process was highly reliable and consistent with previously published standards (Dong *et al.*, 2024). Such data quality

Table 1 Quality control results of *Escherichia coli* isolates from dairy cattle feces collected from dairy farms in Kebon Pedes, Bogor, West Java

Variable	Mean	SE Mean	StDev	Min	Max
Fastq (bp)	207.266.691	10.997.926	42.594.786	148.930.235	329.937.094
Median read length	5.456	243	943	4.157	6.974
Mean read length	7.528	234	907	5.436	8.763
N50 (bp)	4.450	339	1.313	2.800	6.553
Completeness (%)	94,1	0,6	2,3	90,9	97,3
Contamination (%)	2,9	0,4	1,6	0,5	4,8

Mean: average value, SE mean: standard error of the mean, StDev: standard deviation, Min: Minimum, Max: Maximum, bp: base pair.

Table 2 Distribution of quinolone antibiotic resistance genes in dairy farms in Kebon Pedes, Bogor, West Java

Resistance gene	Dairy Farm ID															%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
qnrS1_1	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	100
qnrVC4_1	○	●	○	●	●	●	●	○	○	○	○	○	○	○	○	33

Detected (●), not detected (○).

ensured that subsequent bioinformatic analyses could be conducted with confidence, minimizing the risk of bias or assembly artifacts.

Distribution of quinolone resistance genes

Analysis of the assembled sequences revealed the presence of quinolone resistance genes in *E. coli* isolates from all surveyed dairy farms. Two plasmid-mediated quinolone resistance (PMQR) genes, *qnrS1_1* and *qnrVC4_1*, were successfully identified (**Table 2**). The *qnrS1_1* gene was consistently detected in all isolates (100%), representing the dominant genotype across the sampling sites. Meanwhile, *qnrVC4_1* was present in five isolates (33%), often in combination with *qnrS1_1*.

Most isolates (67%) harbored a single PMQR gene, whereas the remaining 33% carried both genes concurrently. This pattern suggests that *E. coli* from dairy cattle in the study area likely share a common resistance mechanism associated with *qnrS1_1*, with additional variability contributed by *qnrVC4_1*. The co-occurrence of these genes on plasmids underscores their potential mobility and the possibility of horizontal gene transfer among bacterial populations within the livestock environment.

Discussion

Whole genome sequencing (WGS) using the MinION platform was conducted on 15 *E. coli* isolates obtained from the feces of dairy cows at Kebon Pedes Dairy Farm, Bogor City, to identify quinolone resistance genes. The MinION platform was selected because its long-read sequencing technology provides comprehensive genetic information (Chen *et al.*, 2023) and enables accurate resolution of both plasmid and chromosomal structures (Espinosa *et al.*, 2024). With these advantages, the MinION platform facilitates rapid and detailed identification of antimicrobial resistance genes with high precision.

The sequencing results revealed that all *E. coli* isolates (100%) obtained from the 15 farms harbored quinolone resistance genes, suggesting a considerable risk of quinolone antibiotic resistance gene (ARG) dissemination in the surrounding environment. Molecular analysis confirmed the presence of *qnrS1_1* and *qnrVC4_1* genes, both of which are classified as plasmid-mediated quinolone resistance (PMQR) determinants. This finding implies that horizontal transfer of ARGs via plasmids presents a greater risk of dissemination than vertical transfer through chromosomal inheritance.

According to Jeong *et al.* (2008), the presence of two or more resistance genes on a single plasmid can significantly increase quinolone resistance levels. The qnr genes are known to be distributed among a variety of plasmid types, which facilitates their rapid spread within bacterial populations. Plasmid-borne resistance genes can disseminate efficiently owing to the autonomous replication capacity of plasmids, circular DNA molecules typically ranging from 1 to 500 kb, and their ability to transfer between cells through conjugation (Acquaah, 2004). In addition, plasmids can function as vectors for specific genes, including those conferring antibiotic resistance, thereby providing a selective advantage to their bacterial hosts, especially under antibiotic pressure (Casali & Preston, 2003).

The distribution of antibiotic resistance genes across the 15 farms, as shown in **Table 2**, revealed that one gene, *qnrS1_1*, was predominant and detected in all sampling locations. The dominance of a single gene suggests differences in the dissemination dynamics among the various resistance genes. According to Huang *et al.* (2012), the *qnrS1* gene is commonly found in *E. coli* strains resistant to quinolones. The *qnrS* gene, which originates from an IncX-type plasmid capable of conjugation (Slettemeås *et al.*, 2019), is frequently associated with mobile genetic elements that serve as major vehicles for horizontal gene transfer within the Enterobacteriaceae family. This association likely explains the widespread detection of the *qnrS1_1* gene across all farms, as the high mobility of IncX-type plasmids facilitates its broad distribution and directly connects the genetic mechanism to the observed epidemiological pattern (Rozwandowicz *et al.*, 2018; Carattoli, 2023). IncX plasmids are known to play a key role in the dissemination of quinolone resistance owing to their strong capacity for intercellular transfer among bacterial populations.

In contrast, the qnrVC gene is typically found within a cassette array of class 1 integrons, which are genetic elements that promote the acquisition and expression of multiple antibiotic resistance genes (Wu *et al.*, 2012). However, previous studies have also reported the presence of *qnrVC* in IncP-6 plasmids (Zhao *et al.*, 2024), which are generally considered non-conjugative. This implies that the transfer of *qnrVC* requires the involvement of additional mobilization elements or alternative mechanisms of gene exchange. Such genetic complexity likely contributes to the lower prevalence of *qnrVC4_1*, as its mobilization process is more intricate compared to *qnrS1_1* (Fonseca *et al.*, 2008).

Research on quinolone resistance genes in dairy cattle, particularly in Indonesia, remains limited. Compared with other antibiotic resistance genes, quinolone resistance genes in *E. coli* isolated from dairy cattle are less frequently reported. A study by Masse *et al.* (2023) identified five quinolone resistance genes (*gyrA*, *parC*, *parE*, *qnrB*, and *qnrS*), in *E. coli* isolates from dairy cattle feces. In contrast, the

present study detected only *qnrS* and *qnrVC* genes. These genes, which confer quinolone resistance, have not only been identified in dairy cattle but also reported in other livestock species, including pigs (*qnrS* and *qnrVC*) (Latif *et al.*, 2024) and poultry (*qnrA*, *qnrB*, and *qnrS*) (Kurnia *et al.*, 2018; Palupi *et al.*, 2023).

The type of antibiotic resistance gene (ARG) identified in *E. coli* isolates from dairy cattle feces in this study is associated with potential quinolone resistance, particularly against ciprofloxacin. Interestingly, ciprofloxacin is not used as a feed additive and is rarely administered in dairy cattle farms. Ciprofloxacin, a fluoroquinolone antibiotic, is mainly used in human medicine, especially for treating urinary tract infections (Nurjanah *et al.*, 2020). In livestock, particularly dairy cattle, quinolone antibiotics such as enrofloxacin are commonly used to manage bacterial infections related to mastitis, respiratory diseases, and gastrointestinal disorders (Mitchell, 2006). Lin *et al.* (2017) reported an increase in ciprofloxacin-resistant *E. coli* strains in pigs treated with enrofloxacin. Similarly, Kurnia *et al.* (2018) observed that in poultry production systems, antibiotics such as amoxicillin, oxytetracycline, and enrofloxacin are widely used. However, their study revealed that resistance to ciprofloxacin (52%) was higher than resistance to enrofloxacin and norfloxacin (36%). Furthermore, Kaspersen *et al.* (2020) demonstrated that monotherapy with enrofloxacin significantly increases the likelihood of developing quinolone resistance in commensal *E. coli* strains.

This phenomenon can be atrributed to the metabolic conversion of enrofloxacin within the animal body. During hepatic metabolism, enrofloxacin undergoes deethylation of the ethyl group on the piperazine ring, resulting in ciprofloxacin as its main active metabolite (Guo *et al.*, 2014). The detection of ciprofloxacin resistance genes in livestock isolates, despite the exclusive use of enrofloxacin in veterinary practice, highlights an important One Health connection. Enrofloxacin, as a fluoroquinolone antibiotic, is biotransformed into ciprofloxacin in animal tissues, and both compounds share close structural and functional similarities (Martinez *et al.*, 2006). This metabolic relationship provides a plausible explanation for the observed cross-resistance, where the selective pressure exerted by enrofloxacin can co-select for resistance genes such as *qnrS1_1*. This gene confers decreased susceptibility not only to enrofloxacin but also to ciprofloxacin, a critical antimicrobial used in human medicine (Poirel *et al.*, 2012).

Collectively, these findings demonstrate how antimicrobial use in livestock can directly shape resistance patterns with implications for human health, emphasizing the need for integrated surveillance and responsible antimicrobial stewardship within the One Health framework. The results also reaffirm that antibiotic resistance is a cross-species issue that can spread among different livestock populations and ultimately affect humans. The dissemination of resistance genes in dairy farming environments is not driven solely by antibiotic usage but can also occur through environmental contamination or close contact between humans, livestock, and wildlife (Cristóbal-Azkarate *et al.*, 2014). Poor livestock waste management practices can further promote the spread of resistance genes via contaminated fecal matter (Tian *et al.*, 2021).

This is largely attributed to the remarkable capacity of *E. coli* to transmit resistance genes through both horizontal and vertical gene transfer mechanisms (Yunindika *et al.*, 2022). Furthermore, *E. coli* can acquire and maintain resistance genes, serving as a reservoir that facilitates the transfer of these genes to other bacterial species. Once expressed, such transferred genes can lead to the emergence of phenotypic resistance (Abdelfattah *et al.*, 2025). Resistant *E. coli* strains can be transmitted to humans through contaminated animal products or environmental exposure to animal feces, posing serious challenges to both veterinary and human healthcare systems, and representing a growing public health concern (Liu *et al.*, 2020). Inadequate hygiene in animal housing, feed storage areas, and equipment can further promote the proliferation of *E. coli*. Additionally, the improper handling and spread of contaminated livestock waste can facilitate the transmission of *E. coli* from animals to humans as well as into the surrounding environment (Sarba *et al.*, 2023).

This study, conducted in Kebon Pedes Village, Bogor City, an area characterized by high population density, highlights the elevated risk of antimicrobial resistance gene (ARG) transmission in such settings. In densely populated environments, frequent interactions among humans, livestock, and the surrounding environment increase the likelihood of bacterial exchange and horizontal gene transfer (Petersen & Hubbart, 2021). According to Elsharkawy *et al.* (2024), dairy farms in Kebon Pedes are generally small-scale operations, with livestock populations ranging from approximately 5 to 40 head, and some farms are located adjacent to or integrated with other livestock facilities. These conditions create opportunities for cross-contamination and may intensify the spread of *E. coli* between farms.

Contaminated environments provide favorable conditions for the dissemination of antibiotic resistance genes (Pal *et al.*, 2016). Therefore, the combination of high population density and the close proximity of livestock operations in Kebon Pedes Village not only increases the potential for *E. coli* cross-contamination but also accelerates the spread of antibiotic resistance genes. To mitigate these risks, improvements in livestock management practices are essential. These include proper manure handling and disposal, prudent use and monitoring of antibiotics, and comprehensive farmer education programs. Implementing such measures is crucial to reduce the transmission of antibiotic resistance that poses a threat to human, animal, and environmental health.

Conclusion

All *E. coli* isolates obtained from 15 dairy farms in Kebon Pedes, Bogor City, were found to carry quinolone resistance genes. Two variants, *qnrS1_1* and *qnrVC4_1*, were identified, with *qnrS1_1* being the most prevalent. Both genes were located on plasmids, indicating plasmid-mediated quinolone resistance (PMQR). The detection of these genes in all isolates suggests that dairy cattle feces may serve as an important environmental reservoir for antibiotic resistance genes within

farm settings, posing a potential risk of dissemination to humans and other animal populations.

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Author contribution MAR: Data curation, formal analysis, methodology, and writing – original draft. SE: isolate preparation, methodology, and formal analysis. AI: Supervision, investigation, and writing – review & editing. HL: Conceptualization, supervision, project administration, methodology, validation, and writing – review & editing.

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