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RESEARCH ARTICLE

Whole-genome sequencing reveals antimicrobial resistance genes in commensal *Escherichia coli* from healthy pregnant women living near pig slaughterhouses in Indonesia

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ABSTRACT

Background and Aim: Antimicrobial resistance (AMR) is a growing global health concern, particularly at the human–animal–environment interface where livestock production may facilitate the circulation of antibiotic resistance genes (ARGs). Pig slaughterhouses are recognized as potential reservoirs of resistant bacteria, yet genomic information regarding commensal *Escherichia coli* from healthy human populations living nearby remains scarce in Indonesia. Pregnant women represent an important sentinel population because intestinal carriage of resistant bacteria may contribute to neonatal colonization and early-life infections. This study aimed to characterize ARG profiles and their genomic locations in commensal *E. coli* isolated from healthy pregnant women residing near pig slaughterhouses in Banten Province, Indonesia.

Materials and Methods: Rectal swab samples were collected from 100 healthy pregnant women attending primary healthcare centers located within a 5-km radius of pig slaughterhouses. Ninety-four *E. coli* isolates were recovered and subjected to whole-genome sequencing using the Oxford Nanopore Technologies MinION platform. Bioinformatic analyses were performed to identify ARGs and determine their chromosomal or plasmid localization.

Results: Thirty distinct ARGs belonging to 10 antimicrobial classes were detected. Beta-lactamase genes exhibited the greatest diversity, with chromosomally encoded *blaEC-15*, *blaEC*, and *blaEC-18* predominating. Aminoglycoside resistance genes represented the second most diverse group, whereas plasmid-associated genes such as *sul2*, *qnrS1*, and *tet(A)* were among the most frequently identified mobile resistance determinants. Overall, chromosomal ARGs were more prevalent than plasmid-borne genes. Although most isolates carried resistance determinants affecting fewer than three antimicrobial classes, 12.8% exhibited potential multidrug resistance. Shared plasmid-mediated ARGs previously reported in livestock reservoirs from the same region suggested localized circulation of resistance elements across sectors.

Conclusion: Commensal *E. coli* from healthy pregnant women living near pig slaughterhouses harbored diverse ARGs, predominantly chromosomal beta-lactamase genes, together with several plasmid-mediated determinants shared with livestock-associated reservoirs. These findings highlight the interconnected nature of AMR dissemination and support the incorporation of pregnant women into integrated One Health surveillance programs. Genome-based monitoring may facilitate early detection of emerging resistance and guide region-specific interventions to mitigate AMR spread.

Keywords: antibiotic resistance genes, antimicrobial resistance, commensal *Escherichia coli*, multidrug resistance, One Health, pig slaughterhouses, pregnant women, whole-genome sequencing.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most critical threats to global health, food security, and socioeconomic development [1–3]. The dissemination of antibiotic resistance genes (ARGs) among

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bacterial populations, particularly *Escherichia coli*, poses a major challenge in both clinical and environmental settings [4]. As a commensal inhabitant of the gastrointestinal tract of humans and animals [5, 6], *E. coli* is widely used as an indicator organism for monitoring AMR because of its capacity to acquire, maintain, and disseminate resistance determinants [7, 8].

Pigs constitute one of the most important livestock species worldwide [9], and intensive pig production has been closely associated with the emergence and spread of AMR [10]. More than 60% of the global pig population is concentrated in Asia, particularly in East and Southeast Asia, where antimicrobials are extensively used for disease prevention and growth promotion [11]. In Indonesia, Banten Province represents one of the regions with a considerable pig production sector, with approximately 4,924 pigs and 10 pig slaughterhouses recorded in 2022. Previous genomic investigations conducted in Banten Province have characterized antibiotic-resistant *E. coli* isolated from pig farms and slaughterhouses, revealing diverse plasmid-mediated ARGs and emphasizing the importance of these environments as reservoirs of resistance determinants [12, 13]. Nevertheless, the corresponding human interface within the same ecological setting has received limited attention, hindering a comprehensive understanding of the transmission continuum linking livestock and human populations.

The close interaction among humans, pigs, and the surrounding environment facilitates the exchange of bacterial strains and ARGs, thereby contributing to the regional burden of AMR [14]. Although increasing attention has been directed toward AMR at the human–animal–environment interface, information regarding the molecular characteristics of ARGs in *E. coli* isolated from healthy human carriers living near pig-associated environments remains scarce, particularly in Southeast Asia. Pregnant women represent a unique and potentially vulnerable population because intestinal carriage of resistant *E. coli* may contribute to neonatal colonization and increase the risk of early-onset infections, including neonatal sepsis. Consequently, surveillance of AMR at the human–animal–environment interface is essential for elucidating the mechanisms underlying resistance dissemination and identifying populations that may serve as reservoirs of resistant bacteria [15].

Recent advances in portable sequencing technologies have provided new opportunities for genome-based AMR surveillance. The Oxford Nanopore Technologies (ONT) MinION platform enables rapid and high-resolution characterization of resistance determinants, making it particularly suitable for surveillance activities in resource-limited settings and among non-clinical populations. In accordance with recommendations from the World Health Organization (WHO) and the Global Antimicrobial Resistance and Use Surveillance System (GLASS), whole-genome sequencing (WGS) offers enhanced discriminatory power for investigating resistance mechanisms and transmission dynamics [16]. Furthermore, the WHO Tricycle framework has highlighted the importance of integrated One Health surveillance involving humans, animals, and the environment [17].

Despite previous genomic studies demonstrating the occurrence of diverse plasmid-mediated ARGs in *E. coli* isolated from pig farms and slaughterhouses in Banten Province [12, 13], comparable genomic information from humans residing in the same geographic area remains largely unavailable. Most existing studies have focused either on livestock-associated reservoirs or on clinical human isolates, whereas healthy community carriers have been insufficiently investigated. In particular, pregnant women have received little attention despite their potential role as sentinel populations and the possibility of vertical transmission of resistant bacteria to neonates. Moreover, few studies have simultaneously explored the genomic localization of resistance determinants, distinguishing chromosomal from plasmid-mediated ARGs, which is essential for understanding the persistence and mobility of resistance elements. Consequently, substantial knowledge gaps remain regarding the resistome composition of commensal *E. coli* in healthy pregnant women living near pig slaughterhouses and the potential genomic overlap between human and livestock reservoirs within a shared ecological setting.

Therefore, this study aimed to characterize the resistome of commensal *E. coli* isolated from healthy pregnant women residing near pig slaughterhouses in Banten Province, Indonesia, using WGS based on the ONT MinION platform. Specifically, this study sought to determine the diversity and distribution of ARGs, identify their chromosomal and plasmid localization, evaluate the potential occurrence of multidrug resistance (MDR) profiles, and provide insights into the genomic relationship between human and livestock-associated resistance reservoirs within a One Health framework. Through genome-based surveillance, this study provides baseline information that may support integrated AMR monitoring and facilitate the development of region-specific interventions aimed at mitigating the spread of AMR.

MATERIALS AND METHODS

Ethical approval

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki

and applicable national guidelines for research involving human participants. The study protocol, including sample collection procedures, participant recruitment, and data handling methods, was reviewed and approved by the Ethics Committee for Research Involving Human Subjects, IPB University, Bogor, Indonesia (Ethical Approval No. 1382/IT3.KEPMSM-IPB/SK/2024). Prior to enrollment, all participants were informed about the objectives and procedures of the study, and written informed consent was obtained from each participant. Participation was voluntary, and participants were free to withdraw from the study at any stage without any consequences. To ensure privacy and confidentiality, all samples and associated data were anonymized and handled exclusively for research purposes. The collection, transportation, processing, and analysis of biological specimens were performed following established biosafety and ethical standards to safeguard both participants and researchers. No invasive procedures beyond routine rectal swab collection were performed, and no personal identifiers were disclosed during data analysis or publication.

Study period and location

The study was conducted from August 2024 to October 2025 in Banten Province, Indonesia. Sample collection was performed in communities located near pig slaughterhouses, and subsequent laboratory and genomic analyses were conducted using established microbiological and molecular approaches.

Study design

This cross-sectional study was designed to investigate the distribution and genomic characteristics of ARGs in commensal *E. coli* isolated from healthy pregnant women residing near pig slaughterhouses in Banten Province, Indonesia. Healthy pregnant women attending antenatal care services at primary healthcare centers located within a 5-km radius of pig slaughterhouses were included in the study. Isolates recovered from rectal swab samples were subjected to bacterial isolation, DNA extraction, WGS, and bioinformatic analyses to determine the diversity and genomic localization of ARGs. Furthermore, the generated genomic data were intended to facilitate future comparative analyses with isolates previously obtained from pig farms and slaughterhouses in the same region within a One Health framework.

Sample collection and preparation

Rectal swab samples were collected from 100 healthy pregnant women and initially cultured on eosin methylene blue agar before further processing according to the WHO Tricycle framework guidelines [17]. However, the microbiological approach was modified to include general *E. coli* isolates rather than selective screening for extended-spectrum β-lactamase (ESBL)-producing strains. The participants were healthy pregnant women attending antenatal care services at primary healthcare centers (Pusat kesehatan masyarakat/Puskesmas) located within a 5-km radius of a pig slaughterhouse [18].

This community-based sampling strategy in antenatal care settings near livestock-associated environments provides a practical framework for integrating AMR surveillance into routine maternal health services while simultaneously capturing a high-exposure human sentinel population within a One Health context. *E. coli* was identified and isolated from 94 of the 100 rectal swab samples.

DNA extraction and DNA quality control

Genomic DNA was extracted from cultured *E. coli* isolates using the PowerWater DNA Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and quality were subsequently measured using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

WGS

In accordance with the recommendations of WHO and GLASS [16], WGS was performed using the ONT MinION platform. The ONT MinION platform enables rapid, real-time, genome-based AMR surveillance in resource-limited settings and facilitates its application in non-clinical populations such as healthy carriers.

Purification and barcoding of DNA libraries were performed using the Rapid Sequencing gDNA Barcoding Kit (SQK-RBK110.96; Oxford Nanopore Technologies, Oxford, UK) according to the manufacturer's instructions.

Bioinformatic analysis

FASTQ reads generated by the MinION platform were processed in a Linux-based environment. High-quality long reads were selected using Filtlong (v0.2.1) with the parameters --min_length 1000 and --keep_percent 90, and read statistics were subsequently evaluated using NanoStat (v1.6.0).

Genome assembly was performed using Flye (v2.9.5-b1801), followed by polishing with Medaka (v2.0.1) and

Homopolish (v0.4.1). Assembly performance, including genome completeness and contamination, was assessed using QUAST (v5.3.0) and CheckM (v1.2.3). This combined long-read assembly and polishing workflow was optimized for ONT data to improve genome accuracy and facilitate reliable identification of ARGs and their genomic locations (chromosomal versus plasmid).

The assembled contigs were analyzed for ARGs using AMRFinderPlus (v4.0.19) with the National Center for Biotechnology Information database, whereas plasmid replicons were identified using PlasmidFinder (v2.1.6). Integration of ARG detection and plasmid replicon analyses enabled location-specific mapping of resistance genes. The abundance and distribution of ARGs in each isolate were further characterized through bioinformatic analyses.

In addition, the generated genomic data were designed to be directly comparable with previously collected isolates from pig farms and slaughterhouses in the same region, thereby enabling future integrated comparative analyses and contributing to the development of a regional AMR genomic database.

Data visualization

All charts presented in this study were generated using Canva (Canva Pty Ltd., Sydney, Australia) to ensure visual clarity and consistency. The figure illustrating the distribution of ARGs according to antibiotic class and genomic location (plasmid or chromosome) was generated in R (v4.5.0) through RStudio (v2025.05.0+496; Posit Software PBC, Boston, MA, USA) using the ggsankey package to depict the relationships and flow patterns between resistance gene classes and their genomic locations.

RESULTS

Sequencing quality assessment

The quality assessment of WGS data generated from 94 *E. coli* isolates recovered from healthy pregnant women residing near pig slaughterhouses in Banten Province, Indonesia, demonstrated excellent sequencing performance (Table 1), with genome completeness exceeding 90% and contamination levels below 5% [19, 20]. These findings indicate that the sequencing data were representative and of sufficient quality for subsequent bioinformatic analyses. Overall, the high-quality sequencing output provided a reliable basis for characterizing the distribution and genomic localization of ARGs.

Table 1: Quality assessment results of *Escherichia coli* sequencing.

Variables	Total isolates	Mean	Standard error of the mean	Standard deviation	Minimum	Maximum
FASTQ	94	591,499,255	32,475,989	314,866,395	37,029,655	1,322,401,592
Median read length	94	6,532	197	1,590	2,829	10,472
Mean read length	94	9,082	246	1,981	3,910	13,162
Genome size	94	4,879,822	20,792	198,341	4,570,881	5,631,841
N50	94	4,549,428	88,404	843,318	145,524	5,335,530
Completeness	94	99.0	0.1	1.2	91.5	99.6
Contamination	94	1.1	0.1	0.6	0.2	4.1

FASTQ = File format containing raw sequencing reads, N50 = Length of the shortest contig for which 50% of the total genome assembly is represented by contigs of equal or greater length.

Distribution of args among *E. coli* isolates

Diversity of ARGs: Analysis of ARG profiles revealed a total of 30 distinct ARGs distributed among the isolates (Table S1). These genes were classified into 10 antimicrobial classes, namely aminoglycosides, beta-lactamases, chloramphenicol, lincosamides, macrolides, quinolones, streptothricins, sulfonamides, tetracyclines, and trimethoprim (Table 2).

The beta-lactamase class exhibited the highest diversity, comprising 10 ARG variants (Figure 1). Among these, *blaEC-15* was the most frequently detected gene, occurring in 41.5% of isolates, followed by *blaEC* (28.7%) and *blaEC-18* (19.1%). Aminoglycoside resistance genes represented the second most diverse class, with seven ARG variants. Among them, *aph(3'')-Ib* and *aph(6)-Id* were each identified in 6.4% of isolates (Figure 2). The remaining antimicrobial classes contained between one and three ARG variants. Overall, *blaEC-15* was the most prevalent resistance determinant detected and belonged to the beta-lactamase class (Table S1).

Genomic localization of ARGs: Analysis of ARG localization revealed that resistance genes were more frequently

detected on chromosomes than on plasmids, indicating that chromosomes constitute the major reservoir of ARGs in these *E. coli* isolates (Figure 3). A total of 23 distinct ARG variants distributed across 10 antimicrobial classes were identified on chromosomes (Table 2). Among these, *blaEC-15*, *blaEC*, and *blaEC-18* were the predominant beta-lactamase genes.

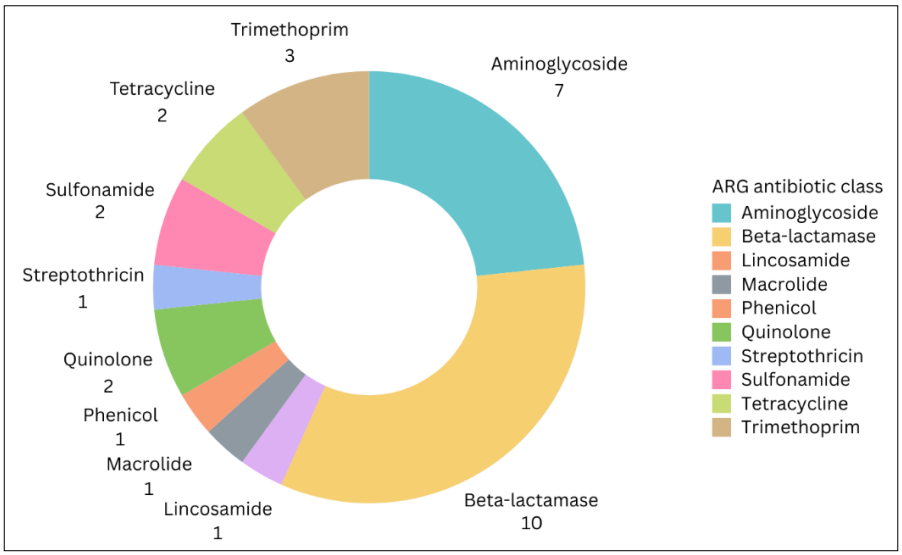

Figure 1: Number of antibiotic resistance gene variants detected across antimicrobial classes. A total of 10 antimicrobial classes were identified. Beta-lactamase resistance genes exhibited the greatest diversity, comprising 10 variants, followed by aminoglycoside resistance genes with seven variants.

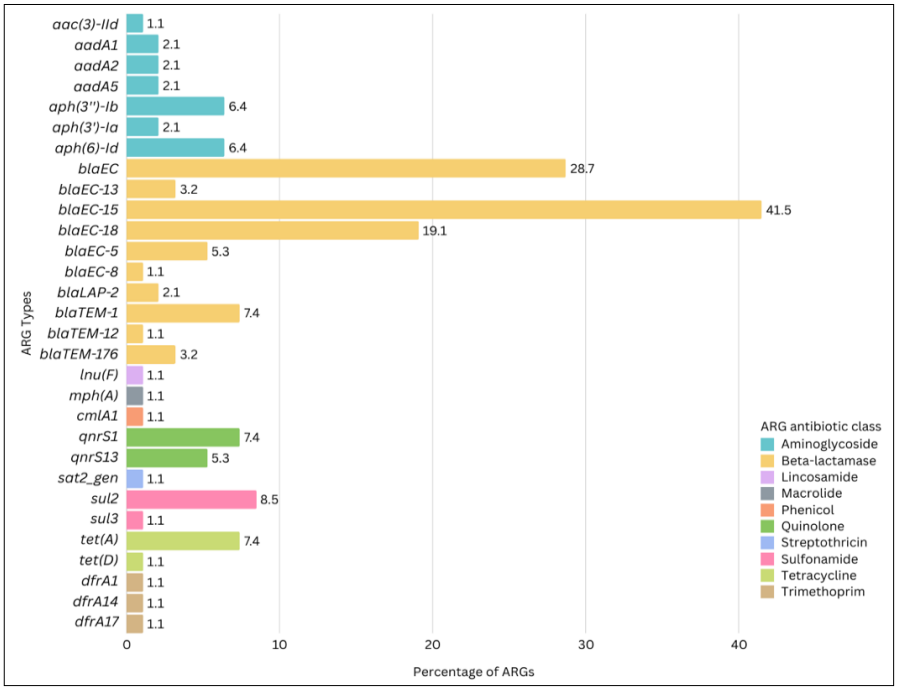

Figure 2: Distribution of antibiotic resistance genes among all isolates. The most frequently detected gene was *blaEC-15*, followed by *blaEC-18* and *blaEC*, all of which belong to the beta-lactamase class.

In contrast, plasmid-associated ARGs were distributed among seven antimicrobial classes, with comparable diversity observed in beta-lactamase and aminoglycoside resistance genes. The most prevalent plasmid-borne genes were *sul2* (sulfonamide class), *qnrS1* (quinolone class), and *tet(A)* (tetracycline class). Overall, plasmids carried a broader range of ARGs spanning multiple antimicrobial classes (Figure 4).

Potential MDR

Most isolates (87.2%) harbored ARGs conferring resistance to fewer than three antimicrobial classes (Table 3). In contrast, 12.8% of isolates exhibited potential MDR, defined as resistance to three or more antimicrobial classes. Specifically, 11.7% of isolates demonstrated resistance to three to six antimicrobial classes, whereas one isolate (1.1%) carried ARGs conferring resistance to more than six antimicrobial classes.

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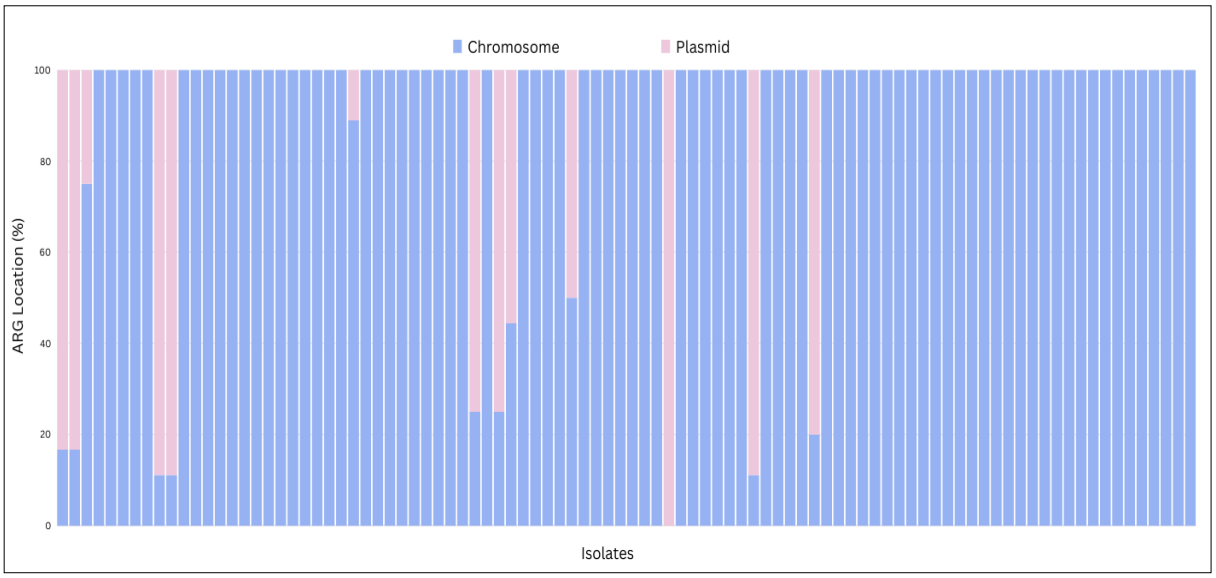

Figure 3: Distribution of antibiotic resistance genes located on chromosomes (blue) and plasmids (pink) among 94 isolates, demonstrating a predominance of chromosomal antibiotic resistance genes.

Table 2: Distribution of antibiotic resistance genes according to chromosomal and plasmid locations.

Antibiotic classes	ARGs	Chromosome (*n*)	Chromosome (%)	Plasmid (*n*)	Plasmid (%)
Aminoglycoside	*aac(3)-IId*	1	0.88	0	0.00
	aadA1	2	1.77	0	0.00
	aadA2	2	1.77	0	0.00
	aadA5	0	0.00	2	3.92
	aph(3')-Ia	0	0.00	2	3.92
	aph(3'')-Ib	1	0.88	5	9.80
	aph(6)-Id	1	0.88	5	9.80
Beta-lactamase	*blaEC*	27	23.89	0	0.00
	blaEC-13	3	2.65	0	0.00
	blaEC-15	39	34.51	0	0.00
	blaEC-18	18	15.93	0	0.00
	blaEC-5	5	4.42	0	0.00
	blaEC-8	1	0.88	0	0.00
	blaLAP-2	0	0.00	2	3.92
	blaTEM-1	2	1.77	5	9.80
	blaTEM-12	0	0.00	1	1.96
	blaTEM-176	1	0.88	2	3.92
Chloramphenicol	*cmlA1*	1	0.88	0	0.00
Lincosamide	*lnu(F)*	1	0.88	0	0.00
Macrolide	*mph(A)*	0	0.00	1	1.96
Quinolone	*qnrS1*	1	0.88	6	11.76
	qnrS13	0	0.00	5	9.80
Streptothricin	*sat2_gen*	1	0.88	0	0.00
Sulfonamide	*sul2*	1	0.88	7	13.73
	sul3	1	0.88	0	0.00
Tetracycline	*tet(A)*	1	0.88	6	11.76
	tet(D)	1	0.88	0	0.00
Trimethoprim	*dfrA1*	1	0.88	0	0.00
	dfrA14	1	0.88	1	1.96
	dfrA17	0	0.00	1	1.96
Total		113	100.00	51	100.00

ARGs = Antibiotic resistance genes. *n* represents the number of ARG detection events (gene occurrences), and % represents the proportion relative to total ARG occurrences within each antimicrobial class.

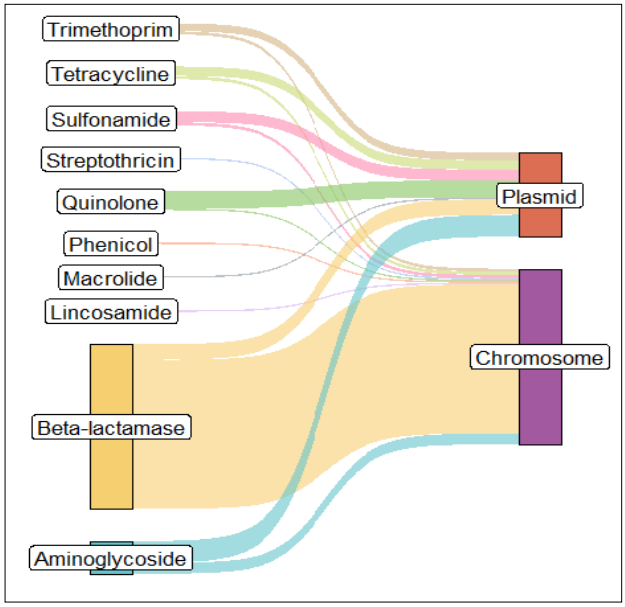

Figure 4: Distribution of antibiotic resistance genes (ARGs) among antimicrobial classes according to chromosomal and plasmid locations. A greater diversity of antimicrobial classes was observed on plasmids, whereas chromosomes contained the highest number of ARG variants, primarily belonging to the beta-lactamase class. Several antimicrobial classes contained ARGs located on both chromosomes and plasmids.

Table 3: Potential multidrug resitance profiles.

Number of antimicrobial classes resistant	Number of isolates	% of total (n = 94)
<3 classes	82	87.2
3–6 classes	11	11.7
≥7 classes	1	1.1
Total	94	100.0

DISCUSSION

Principal findings

This study investigated the genomic resistome of *E. coli* at the human–animal interface in a pig-dense slaughterhouse area in Banten Province, Indonesia, using WGS. To the best of our knowledge, this represents the first WGS-based resistome analysis of *E. coli* isolated from healthy pregnant women residing in this high-risk interface area in Indonesia.

A major finding of this study was the identification of a distinct genomic architecture of AMR, characterized by the predominance of chromosomally encoded *blaEC* family genes in human isolates. This pattern contrasts with the plasmid-dominated ARG profiles previously reported in pigs from the same region [12, 13], suggesting host-associated evolutionary structuring and potentially reduced horizontal gene transfer in human commensal *E. coli*. In addition, this study provides genomic evidence of shared plasmid-mediated ARGs, including *sul2*, *qnrS1*, and *tet(A)*, among humans, pigs, and slaughterhouse environments within the same province, indicating localized interspecies circulation of resistance determinants under a One Health framework. Furthermore, long-read sequencing using the ONT platform enabled accurate discrimination between chromosomal and plasmid-borne ARGs, thereby overcoming limitations associated with short-read sequencing approaches.

Beta-lactamase resistance genes

The beta-lactamase class represented the most diverse group of ARGs identified in this study, with *blaEC-15*, *blaEC*, and *blaEC-18* being the predominant variants, all of which were chromosomally encoded. These genes belong to the *blaEC* family, which encodes class C beta-lactamases, also known as AmpC-type enzymes [21]. AmpC beta-lactamases confer resistance by hydrolyzing broad- and extended-spectrum cephalosporins, including cephamycins and oxyimino-beta-lactams, and are not inhibited by beta-lactamase inhibitors such as clavulanic acid [22]. Members of the *blaEC* family, including *blaEC-5*, *blaEC-8*, *blaEC-15*, *blaEC-16*, *blaEC-18*, and *blaEC-19*, have been characterized as serine beta-lactamases with substrate specificity toward cephalosporins [23]. Among these, the *blaEC* family represents an important group of resistance determinants frequently encountered in *E. coli* [24, 25].

In the present study, the predominance of *blaEC-15*, *blaEC*, and *blaEC-18*, all corresponding to chromosomally encoded AmpC β-lactamases [26], revealed a resistome profile distinct from those reported globally and in many low- and middle-income countries, where acquired ESBL genes such as *blaCTX-M* are the

major determinants of β-lactam resistance in human carriage populations [14]. This pattern may reflect intrinsic chromosomal AmpC activity and local selective pressures favoring *blaEC* genes over plasmid-borne ESBL determinants. Moreover, the predominance of chromosomal *blaEC* genes in human isolates, compared with plasmid-mediated ARGs reported in livestock-associated *E. coli*, suggests differences in ecological pressures, with more stable intrinsic resistance in humans and greater horizontal gene transfer occurring in pigs.

The chromosomal localization of these genes, particularly *blaEC-5* and *blaEC-18*, is consistent with previous studies describing the integration of AMR genes into bacterial chromosomes. This phenomenon highlights the dynamic mobility of resistance determinants, which can move between mobile genetic elements, such as plasmids and integrons, and chromosomal DNA. Apoorva *et al.* [22] emphasized this bidirectional transfer mechanism and demonstrated that resistance genes may shift from plasmids to chromosomes and vice versa, thereby facilitating their persistence and dissemination even in the absence of selective antimicrobial pressure.

In the present study, *blaEC* genes were detected not only in human isolates but also in pig isolates collected from areas surrounding pig slaughterhouses [27]. This observation agrees with findings from a systematic review by Iryawati *et al.* [14], which reported a predominantly human-associated distribution of *blaEC* genes in *E. coli* at the human–pig interface. The WGS evidence obtained in the present study extends these observations by confirming the genomic context and chromosomal localization of *blaEC* genes across both human and pig reservoirs within a shared ecological setting. These similarities suggest that host-specific selection pressures and differences in antimicrobial exposure may influence the distribution of *blaEC* genes among reservoirs.

Aminoglycoside resistance genes

Following beta-lactamase genes, aminoglycoside resistance genes represented the second most diverse class detected in this study. Among these, *aph(3'')-Ib* and *aph(6)-Id* were the most frequently identified variants and were predominantly located on plasmids. These genes encode aminoglycoside O-phosphotransferases, which constitute the major mechanism of aminoglycoside resistance in clinical settings [28]. The products encoded by *aph(3'')-Ib* and *aph(6)-Id* enzymatically inactivate aminoglycosides through phosphorylation, thereby preventing their interaction with bacterial target sites [29, 30].

Notably, *aph(3'')-Ib* (also referred to as *strA*) and *aph(6)-Id* (*strB*) frequently occur as a gene pair on mobile genetic elements and have been identified in a broad range of bacterial pathogens from plants, animals, and humans [31]. Their plasmid-mediated nature facilitates horizontal transfer and persistence across different ecological reservoirs. Previous studies conducted in Banten Province demonstrated that these genes were predominant among *E. coli* isolates originating from pig farms [13]. Subsequent transmission to pig slaughterhouses was also observed, although with lower frequencies [27]. These findings support the present results and highlight the potential for cross-species dissemination of aminoglycoside resistance genes through mobile genetic elements.

Sulfonamide, quinolone, and tetracycline resistance genes

Among plasmid-mediated ARGs, *sul2*, *qnrS1*, and *tet(A)* were among the most frequently detected genes, conferring resistance to sulfonamides, quinolones, and tetracyclines, respectively. The detection of *sul2*, which encodes resistance to folate pathway antagonists [32], is of particular concern because it is associated with enhanced expression of dihydropteroate synthase and is frequently mobilized through plasmids and transposable elements [33]. Together with *sul1* and *sul3*, *sul2* has been strongly associated with mobile genetic elements and highlights livestock environments as potential hotspots for ARG dissemination [34].

Previous studies demonstrated that *sul2* was abundant in pig slaughterhouses in Banten Province and was primarily located on plasmids [27], suggesting environmental and interspecies factors facilitating its mobility. Likewise, *qnrS1*, a plasmid-mediated quinolone resistance gene, showed high prevalence in the present study and has also been reported as one of the dominant ARGs in pig slaughterhouses in the same region [13, 27]. Similarly, *tet(A)* emphasizes the importance of plasmids in ARG dissemination among humans living near pig slaughterhouses and among animal reservoirs. Plasmid-associated *tet(A)* has been frequently linked to resistance in pigs and possesses the capacity for horizontal transfer from *E. coli* to other enteric pathogens, including *Salmonella* [35, 36].

The concurrent detection of identical plasmid-mediated ARGs, namely *sul2*, *qnrS1*, and *tet(A)*, among human carriers in the present study and previously characterized pig and slaughterhouse isolates from the same province represents a novel genomic linkage within a localized One Health system. These findings provide direct genomic evidence for the interspecies circulation of mobile resistance elements within a shared ecological and

geographical interface rather than isolated occurrences in independent reservoirs. Collectively, these observations emphasize the persistence and circulation of plasmid-mediated ARGs across human and animal sectors associated with pig slaughterhouse environments.

Potential for MDR development

Most isolates in this study harbored ARGs conferring resistance to fewer than three antimicrobial classes. However, bacteria exhibiting resistance to at least three different classes of antimicrobials are defined as MDR [37]. Although only a limited number of isolates demonstrated MDR potential, their presence raises important public health concerns because of the possibility of ARG dissemination within the community. Previous studies have shown that pig slaughterhouses in Banten Province harbor *E. coli* isolates with MDR potential, indicating that these facilities may serve as reservoirs of resistance determinants with the capacity for interspecies transmission and environmental persistence [27].

The concurrent detection of resistance genes from multiple antimicrobial classes, including beta-lactamases, aminoglycosides, sulfonamides, quinolones, and tetracyclines, among human and pig-associated *E. coli* highlights the complex and interconnected dynamics of AMR. Although most isolates carried resistance determinants affecting fewer than three antimicrobial classes, the occurrence of MDR strains and mobile genetic elements capable of horizontal transfer raises concerns regarding the persistence and dissemination of resistance determinants across reservoirs. Furthermore, the chromosomal localization of beta-lactamase genes in human isolates, together with the plasmid-mediated dissemination of aminoglycoside and tetracycline resistance genes in pigs and the occurrence of *sul2* and *qnrS1* in multiple ecological niches, reflects the influence of shared antimicrobial use across sectors on the selection and distribution of resistance determinants.

Intersectoral implications of medically important antimicrobials

The antimicrobial classes identified in this study belong to categories considered medically important for both human and veterinary medicine, highlighting their significance in cross-sectoral resistance transmission. Beta-lactam resistance genes were the most frequently detected, followed by aminoglycoside resistance genes. Beta-lactams, tetracyclines, and sulfonamides are categorized as highly important antimicrobials and are widely used in both sectors, necessitating prudent regulation to minimize resistance development. Aminoglycosides are classified as critically important antimicrobials and are recommended primarily for therapeutic applications. Although less frequently detected, quinolones belong to the highest priority critically important antimicrobial category, and their use should be restricted to essential therapeutic indications. These findings emphasize the importance of harmonized antimicrobial stewardship strategies to preserve the effectiveness of existing treatments [37].

The occurrence of resistance genes to these antimicrobial classes in both human- and pig-derived isolates highlights the need for integrated antimicrobial stewardship and surveillance across human and animal health sectors. These findings reinforce the importance of One Health approaches for preserving the therapeutic efficacy of existing antimicrobials and mitigating the progression of AMR. Consequently, the development and enforcement of targeted policies regulating antimicrobial use in medical and veterinary settings are essential to prevent the emergence and cross-sectoral transmission of AMR [38, 39]. Recommended measures include stricter antimicrobial prescription policies and improved hygiene practices [40, 41], promotion of non-antibiotic alternatives in animal husbandry [42, 43], and strengthening integrated surveillance systems to monitor resistance trends [44, 45].

ONE HEALTH IMPLICATIONS

The present study supports a One Health sentinel model in which pregnant women residing near livestock interfaces can serve as early indicators of environmental resistome circulation. The occurrence of shared plasmid-mediated ARGs among humans, pigs, and slaughterhouse environments indicates active local transmission networks, whereas the predominance of chromosomal *blaEC* genes suggests host-adapted persistence in humans.

Fecal carriage of resistant *E. coli*, particularly ESBL-producing strains, among pregnant women represents a critically understudied high-risk condition within AMR surveillance frameworks. These bacteria may be vertically transmitted to neonates, thereby increasing the risk of neonatal sepsis and facilitating the dissemination of resistance genes within healthcare settings [17, 46, 47]. Consequently, pregnant women–neonate dyads constitute an important but underrecognized pathway for the transmission of commensal resistant *E. coli*.

Given the close human–animal interactions occurring in slaughterhouse environments, these settings may

act as hotspots for the exchange and persistence of resistant bacteria. Collectively, the findings support the inclusion of pregnant women living in livestock interface areas as a sentinel population for monitoring community-level resistome dynamics and underscore the importance of integrated One Health surveillance strategies.

CONCLUSION

This study provides the first WGS-based characterization of ARGs in commensal *E. coli* isolated from healthy pregnant women residing near pig slaughterhouses in Banten Province, Indonesia. The findings revealed a diverse resistome comprising 30 ARGs across 10 antimicrobial classes, with beta-lactamase genes as the most diverse group. Chromosomally encoded *blaEC-15*, *blaEC*, and *blaEC-18* predominated, whereas plasmid-mediated *sul2*, *qnrS1*, and *tet(A)* were shared with livestock-associated reservoirs from the same region. Although most isolates carried resistance determinants to fewer than three antimicrobial classes, 12.8% showed potential MDR profiles, indicating the presence of clinically relevant resistance risks in a healthy community population.

These findings have practical One Health implications because pregnant women living near livestock interfaces may serve as sentinel populations for monitoring community-level AMR circulation. The detection of shared plasmid-mediated ARGs across human and animal sectors highlights the need for integrated genomic surveillance, prudent antimicrobial stewardship, improved hygiene practices, and targeted interventions around pig slaughterhouse environments.

A major strength of this study was the use of long-read WGS, which enabled differentiation between chromosomal and plasmid-borne ARGs. However, the study was limited by its cross-sectional design, geographic restriction to one province, and lack of direct transmission analysis among humans, pigs, and environmental sources. Future studies should include longitudinal sampling, larger multisectoral datasets, and comparative genomic analysis across human, animal, and environmental isolates.

In conclusion, commensal *E. coli* from healthy pregnant women near pig slaughterhouses harbors clinically important ARGs, including shared mobile resistance determinants, supporting the need to incorporate human sentinel populations into regional One Health AMR surveillance programs.

DATA AVAILABILITY

The data generated during the study are included in the manuscript.

GENERATIVE AI DECLARATION

The authors declare that generative artificial intelligence tools were used solely to improve language, grammar, and readability during manuscript preparation. All scientific content, data analysis, interpretation of results, and conclusions were developed and verified by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the work presented, and no AI tool was listed as an author.

AUTHORS' CONTRIBUTIONS

DI: Conceived and designed the study, developed the methodology, conducted the investigation, curated and analyzed the data, prepared visualizations, and wrote and revised the manuscript. SA, CB, and HL: Contributed to conceptualization and methodology, supervised the study, and critically reviewed and revised the manuscript. PR: Performed laboratory analyses, provided technical support, and contributed to data acquisition. All authors have read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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