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Molecular Detection of *Escherichia coli* in Blood and Tissue Samples Collected from Broiler Chickens in Different Poultry Farms of West Azerbaijan Province, Iran

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Abstract

Avian pathogenic *Escherichia coli* (APEC) represents a major threat to poultry production and public health due to its high virulence potential and the rising prevalence of antimicrobial resistance. This study investigated the prevalence of antimicrobial resistance and virulence genes among *E. coli* isolates recovered from broiler chickens in the West Azerbaijan Province of Iran. A total of 120 isolates obtained from blood and liver samples were identified using standard bacteriological methods and confirmed by PCR amplification targeting the *16SrRNA* gene. The presence of fluoroquinolone resistance genes (*qnrS* and *qnrB*) and the adhesion-associated virulence gene *fimA* was assessed. Isolates were analyzed according to bird age, season, and geographic region. All isolates (100%) were positive for the *16SrRNA* gene. The overall prevalence of *qnrS* and *qnrB* was 47.56% for each gene, while *fimA* was detected in 29.27% of the isolates. The highest prevalence of *qnrS* (52.63%) and *fimA* (31.58%) occurred in 10-day-old chicks, with a marked decline in *fimA* expression at 30 days of age. Regionally, *qnrB* was significantly more prevalent in the southern region (79.17%) compared with the central (23.81%) and northern (40.54%) regions. Seasonal analysis showed higher detection rates of *qnrS* and *fimA* during winter. The co-occurrence of fluoroquinolone resistance and virulence genes in APEC highlights a critical One Health concern, as these resistance determinants could potentially disseminate to human extraintestinal pathogenic *E. coli*. Continuous molecular surveillance and prudent antimicrobial usage are strongly recommended.

Introduction

The family Enterobacteriaceae is one of the most important bacterial families, encompassing both homotrophic and heterotrophic bacteria. This family comprises several bacterial genera, among which *Escherichia coli* (*E. coli*) is particularly noteworthy. The genus *Escherichia* includes Gram-negative bacteria that are natural inhabitants of the gastrointestinal tract of humans and animals (1). These organisms are bacillus-shaped and Gram-negative bacteria. They have a cell envelope that includes lipopolysaccharides and surface proteins. In addition, they possess an inner membrane composed of phospholipids and membrane proteins

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(2). *Escherichia coli* exhibits a variety of biochemical characteristics that can be used for its identification and differentiation. Some of these characteristics include acid production from glucose and lactose; gas production from glucose, lactose, and sucrose; urease production; indole production; a positive catalase test; and a negative oxidase test. These characteristics can be used as diagnostic methods in medical laboratories and the food industry (3).

Considering that *Escherichia coli* is recognized as an important bacterial pathogen in the poultry industry, causing reduced performance and increased mortality in poultry, the control and prevention of these infections in the poultry industry are crucial to avoid significant economic losses(4). On the other hand, the indiscriminate use of antibiotics has led to the development of multidrug-resistant (MDR) strains in broiler chickens. Given the high cost of phenotypic testing, molecular methods are preferable for detecting antibiotic resistance and biofilm production due to their high accuracy (5).

Avian pathogenic *Escherichia coli* (APEC), one of the main causative agents of bacterial infections in poultry farms worldwide, is responsible for a wide range of diseases in chickens. The APEC can cause several conditions in poultry, including peritonitis, salpingitis, yolk sac infection, cellulitis, colisepticemia, pericarditis, airsacculitis, coligranuloma, and arthritis (6). These infections can lead to high mortality rates, resulting in significant economic losses for the poultry industry. The APEC can enter poultry through various routes, including the oral–fecal route, the respiratory tract, and ascending infection via the cloaca (7). The ascending route is particularly important because it can lead to outbreaks characterized by salpingitis peritonitis syndrome in breeder hens, which may result in reduced egg production and egg quality, increased mortality, and higher costs associated with treatment, culling of birds, and carcass disposal (8, 9).

The APEC can also be transmitted vertically from breeder flocks to their offspring, leading to yolk sac infection, omphalitis, and increased mortality during the first week of life in broiler chicks. The APEC strains exhibit genetic diversity and may possess a range of virulence factors that contribute to their pathogenicity(10). These virulence factors include adhesins, toxins, iron acquisition systems, and serum resistance factors. The APEC cells may also be resistant to multiple antibacterial drugs, which can complicate treatment and control efforts. Prevention and control of APEC infections in poultry farms involve a combination of measures, including proper management practices, biosecurity measures, vaccination, and antimicrobial management. Early detection and prompt treatment of infected birds are also essential to prevent the spread of infection and to reduce economic losses (11).

Materials and Methods

Study area

West Azerbaijan Province is one of the most important livestock hubs in Iran, located in the northwestern part of the country and sharing borders with Turkey, Iraq, and the Republic of Azerbaijan. The province features a diverse climate cold and mountainous in the northern regions, temperate in the central areas, and semi-arid in the south creating favorable conditions for raising various livestock species, particularly broiler chickens and small ruminant flocks. According to the Agricultural Jihad Organization of the province (2024), more than 2,300 industrial poultry farms are active in West Azerbaijan, producing millions of broiler chicks annually(Figure. 1)(12).

Blood and tissue collected sampling

In this study, a total of 380 clinical samples, including 190 liver tissue samples and 190 blood samples, were collected from broiler chickens showing clinical signs of septicemia and colibacillosis in 27 industrial poultry farms located in West Azerbaijan Province. To ensure geographical representativeness, the province was divided into three regions (northern,

central, and southern), and poultry farms were evenly selected from each region (nine farms per region). Random sampling was conducted from all farms registered under the Provincial Veterinary Organization during two seasons of the year (autumn and winter). Upon transfer of the samples to the laboratory, bacteriological culture was performed on enriched media (MacConkey agar and blood agar), and 120 *Escherichia coli* isolates were identified and purified based on morphological and biochemical characteristics, including IMViC tests, oxidase negativity, lactose fermentation, and gas production. The confirmed isolates were subsequently stored for DNA extraction and molecular analyses (11).

Fig. 1. Map of West Azerbaijan Province, Iran, highlighting the three geographic regions (North, Center, and South) from which broiler chicken farms were sampled in the study (12).

DNA extraction from blood and tissue

For the retrieval of bacterial DNA from blood samples, the extraction was carried out using the DNA Extraction Mini Kits (Favorgen, Taiwan). Specifically, bacterial DNA was extracted from the blood samples using the Favorprep Blood/Cultured Cell Genomic DNA Extraction Mini Kit 50 prep, following the instructions from Favorgen, Taiwan. The extractions followed the manufacturer's instructions (13).

For DNA extraction from tissue samples, the tissue (2 gram) was initially washed twice with PBS, dried in the open air, and then rapidly frozen using liquid nitrogen. Once frozen, the tissue was crushed using a scalpel blade and transferred into a 2cc microtube. The extraction process was performed using a column kit (DNA extraction kit from tissue and blood RXNS) provided by Iran Biotechnology Company. To ensure the reliability of the extraction technique and to assess the DNA concentration, a random selection of 10 samples was analyzed using NanoDrop 2000c (from Thermo Scientific, USA). The optical absorption of these samples was measured at a wavelength of 260, and the 260/280 ratio (DNA/protein) was determined as an additional indicator of quality and purity. The avarege purity of the DNA used in this study was 250 micrograms per microliter (12).

Table 1. Primer sequences for detection of *Escherichia coli* genes by PCR**.**

Target gene	Sequence (5′-3′)	Product size (bp)	PCR condition	Gene number
16SrRNA	F:GCAGTGGGGAATATTGCACA R:TCAGATGCAGTTCCAGGTT	286	Initial denaturation: 95 °C for 4.0 min. 38 cysles of: Denaturation at 95 °C for 30 sec anling at 58 for 30 secs, Extension at 72 °C for 60 sec. Final extension: 72 °C for 5.0 min.	PX623152 PX623169
fimA	F: ATGGTGGGACCGTTCACTTT R: TGGAACGGAATGGTGTTGGT	321	Initial denaturation: 95 °C for 4.0 min. 38 cysles of: Denaturation at 95 °C for 45 sec anling at 55 for 45 secs, Extension at 72 °C for 60 sec. Final extension: 72 °C for 7.0 min.	PX789364 PX789365
qnrS	F: ACGACATTCGTCAACTGCAA R: TAAATTGGCACCCTGTAGGC	516	Initial denaturation: 95 °C for 4.0 min. 38 cysles of: Denaturation at 95 °C for 30 sec anling at 58 for 30 secs, Extension at 72 °C for 60 sec. Final extension: 72 °C for 7.0 min.	PX753061 PX753062
qnrB	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469	Initial denaturation: 95 °C for 4.0 min. 38 cysles of: Denaturation at 95 °C for 30 sec anling at 58 for 30 secs, Extension at 72 °C for 60 sec. Final extension: 72 °C for 7.0 min.	PX776841 PX776842

Molecular study PCR method

To investigate the presence of bacterial genomic DNA in the samples, the polymerase chain reaction (PCR) method was utilized. This method was employed for the amplification of genes of interest, with a focus on specific target genes. Variations in gene sequences were leveraged to discern the bacterial species. For this investigation, the PCR method was selected, and the designated temperature program was followed (as outlined in Table 1). Primers described by Qiu et al. (2019) were employed (14).

The PCR procedure was conducted in a 25 µl reaction volume. This solution consisted of 4 µl of template DNA (250 ng, derived from the purity stated earlier), 1 µl for each primer (Table 1), and 12.5 µl of master mix. The remaining volume was filled with sterile distilled water. The thermocycler (Quanta Biotech, UK) was programmed with the PCR conditions listed in Table 1 Subsequently, the PCR products underwent electrophoresis on a 1.5% agarose gel, treated with a DNA safe stain (Labnet, ENDURO, USA).

Sequences and Phylogenetic analysis

A subset of the *Escherichia coli* positive samples was sent to Pishgam Biotechnology Company for Sanger dideoxy sequencing of each gene individually. The resulting sequences were read using the SnapGene software, uploaded to the NCBI database, and subjected to BLAST analysis. Nucleotide sequences of each species from different regions were aligned to identify variable positions. The sequences were uploaded to NCBI to search for the most similar reference sequences, and the COI regions were identified using BLAST available at NCBI. COI sequences of *Escherichia coli* from the GenBank database were then used for phylogenetic analysis. Alignments were manually edited to correct any

misalignments using the Clustal W alignment tool and exported in MEGA and FASTA formats. All obtained nucleotide sequences were deposited in GenBank with assigned accession numbers. Subsequently, phylogenetic relationships were constructed and examined using the Maximum Likelihood (ML) method in MEGA version 11. DNA sequence polymorphism analyses were performed to estimate nucleotide diversity using MEGA 11 and the Blastn software (Figure. 2; 15).

Results

The molecular analysis of 120 *Escherichia coli* isolates obtained from liver and blood samples of broiler chickens in West Azerbaijan Province provides strong evidence of the concurrent dissemination of diagnostic, pathogenic, and antibiotic resistance determinants within this microbial population. All isolates tested positive for the *16S rRNA* gene, confirming their taxonomic affiliation with the genus Escherichia and validating the PCR method and DNA extraction quality. Among the genes analyzed, *qnrS* and *qnrB*, both belonging to the quinolone resistance determining region family, exhibited high prevalence. Age-stratified data indicated that the prevalence of *qnrS* was relatively high at 10 days of age (liver: 44.44%, blood: 52.63%), suggesting early exposure of chicks to selective pressure from quinolones, such as prophylactic enrofloxacin use. By 30 days of age, *qnrS* prevalence decreased in the liver (33.33%) while remaining relatively stable in blood (47.22%), possibly reflecting the transition of infection from local sites (gastrointestinal tract/liver) to systemic circulation and the establishment of resistant strains systemically. The observed resurgence of *qnrS* prevalence after 30 days (liver: 50%, blood: 44.44%) may correspond to renewed quinolone administration during the finisher phase. In contrast, *fimA*, encoding the major subunit of type 1 fimbriae involved in initial adhesion to mannose receptors on mucosal surfaces of the respiratory and urogenital tracts, exhibited a non-linear pattern: prevalence at 10 days of age (liver: 22.22%, blood: 31.58%) declined significantly at 30 days (6.67% and 16.46%, respectively) and increased again in older birds (28.57% and 25.93%). Geographical and seasonal distribution of *qnrB* showed a different pattern: prevalence in the southern region (blood: 79.17%) was markedly higher than in the northern (40.54%) and central regions (23.81%). This may be attributed to differences in management practices (e.g., type and dosage of antibiotics, stocking density, vaccination programs) or environmental factors (e.g., temperature, humidity, water and feed quality). A slight increase in *qnrB* prevalence in winter (blood: 48.89%) compared to autumn (45.95%), along with a similar trend for fimA (31.11% in winter vs. 27.03% in autumn), may indicate heightened bacterial activity during colder seasons, possibly due to thermal stress, reduced ventilation, or increased humidity in poultry houses, which facilitate *E. coli* proliferation and transmission (Table 2).

The proximity of red and green dots in all three trees indicates that the strains isolated from blood and tissue are genetically very similar and have originated from a common source. The red and green samples are in the same clade (evolutionary branch) in all three trees (A, B, C). This indicates that these samples originated from a recent common ancestor. The short branch lengths between the red and green dots indicate low genetic distance and high sequence similarity. These samples are more similar to each other than to other *E. coli* strains in the tree. The numbers on the branches (100, 99, 97) indicate bootstrap support. High values (>95) indicate that this grouping is statistically very reliable. The blood and tissue samples are in a separate group from the other reference *E. coli* strains. This indicates that these strains may belong to a specific lineage or clonal complex. The clinical samples (blood and tissue) are evolutionarily very close to each other and they likely indicate clonal spread of a specific strain in the patient's body.

Discussion

The findings of this study indicate that *Escherichia coli* isolates recovered from the blood and liver of broiler chickens in West Azerbaijan Province carry a significant genetic load of fluoroquinolone resistance genes (*qnrS* and *qnrB*) and the adhesion gene (*fimA*). The 100% prevalence of the *16S rRNA* gene confirms the reliability of the molecular methods and the definitive identity of *E. coli*. Overall, the prevalence of *qnrS* and *qnrB* was reported at 47.56% and 22.44%, respectively, while fimA was found in 29.27% of the isolates a pattern that not only highlights the spread of resistance but also its association with virulence traits. The age-dependent pattern of *qnrS* and *fimA* prevalence in 10-day-old chicks (*qnrS*: 52.63%, *fimA*: 31.58% in blood isolates) compared to the 30-day-old group aligns with findings from studies such as Zhang et al. (2023) and Ye et al. (2025). This pattern is likely driven by early selective pressure from the routine use of fluoroquinolones in the initial days of life a common practice reported in many Iranian broiler farms for sepsis prevention (16, 17).

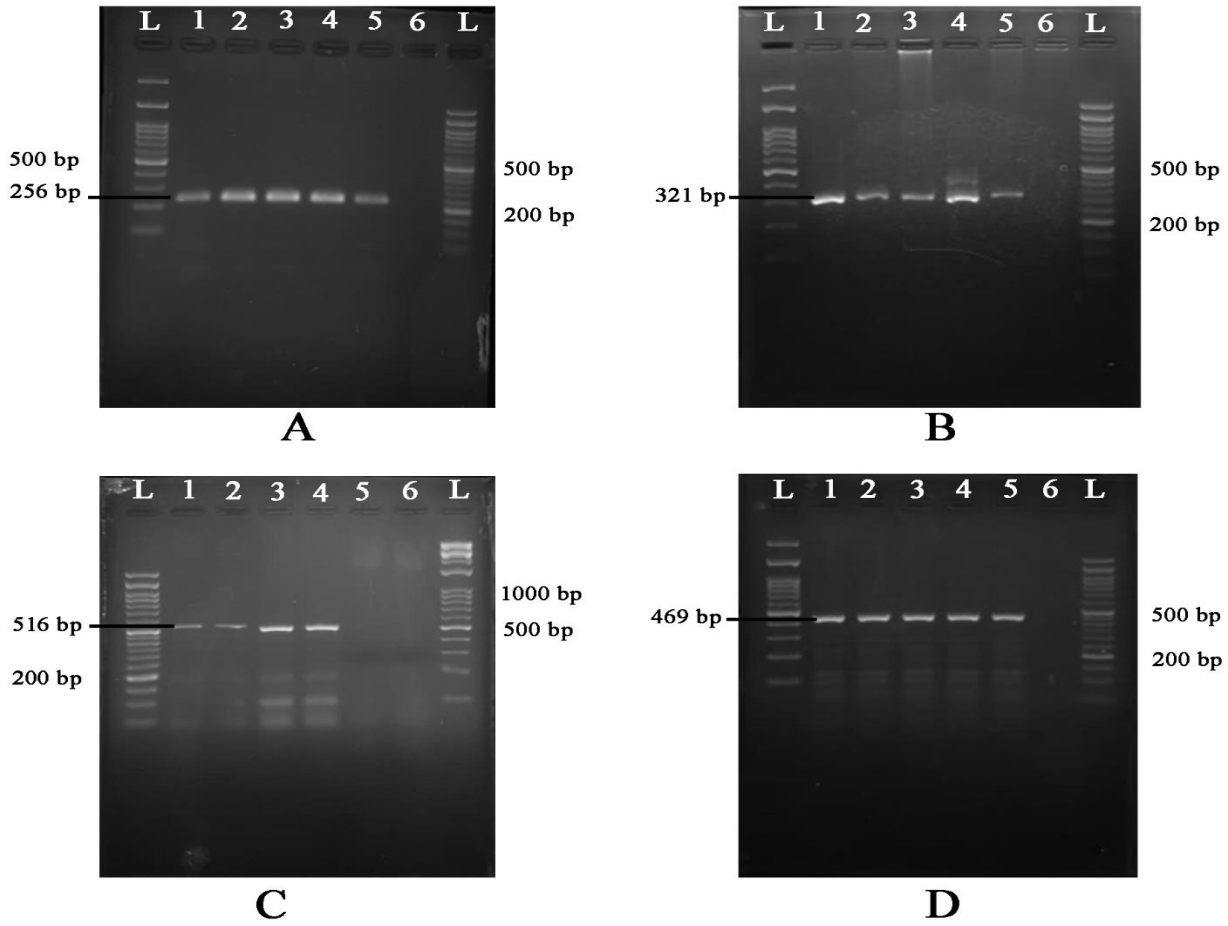

Fig. 2. (A) Agarose gel image of amplified fragment of *E. coli 16SrRNA* gene (256 bp) using PCR. Lane L, 50 and 100-bp molecular ladder (Smobio Technology Inc., Taiwan); lanes 1–5 positive samples for *E. coli*, lane 6, Negative control. **(B)** Agarose gel image of amplified fragment of *E. coli fimA* gene (321 bp) using PCR. Lane L, 50 and100-bp molecular ladders (Smobio Technology Inc., Taiwan); lanes 1–5positive samples for *E. coli* lane 6, Negative control **(C).** Agarose gel image of amplified fragment of *E. coli qnrS* gene (516 bp) using PCR. Lane L, 50 and 100 -bp molecular ladders (Smobio Technology Inc., Taiwan); lanes 1–4 positive samples for *E. coli* lane 6, Negative control **(D).** Agarose gel image of amplified fragment of *E. coli qnrB* gene (469 bp) using PCR. Lane L, 50 and 100 -bp molecular ladders (Smobio Technology Inc., Taiwan); lanes 1–5 positive samples for *E. coli* lane 6, Negative control**.**

Table 2. Prevalence of *E. coli* and its virulence/resistance genes in liver and blood samples from broiler chickens, stratified by age, season, and region.

		Broiler poultry (No.120)			
Gene		*16SrRNA*	*qnrS*	*fimA*	*qnrB*
Age group					
10 days	Liver (9)	9/9 9 (n=9; 100%; 95%Cl: 90.09%-100%)	9/4 9 (n=4; 44.44%; 95%Cl: 18.87%-73.33%)	9/2 9 (n=2; 22.22%; 95%Cl: 6.32%-54.74%)	9/1 9 (n=1; 11.11%; 95%Cl: 1.99%-4.35%)
	Blood (19)	19/19 19 (n=19; 100%; 95%Cl: 83.18%-100%)	19/10 19 (n=10; 52.63%; 95%Cl: 31.71%-72.67%)	19/6 19 (n=6; 31.58%; 95%Cl: 15.37%-53.99%)	19/2 19 (n=2; 10.53%; 95%Cl: 2.94%-3.14%)
30 days	Liver (15)	15/15 15 (n=15; 100%; 95%Cl: 79.61%-100%)	15/5 15 (n=5; 33.33%; 95%Cl: 15.17%-58.28%)	15/1 15 (n=1; 6.67%; 95%Cl: 1.19%-29.82%)	15/1 15 (n=1; 6.67%; 95%Cl: 1.19%-29.82%)
	Blood (36)	36/36 36 (n=36; 100%; 95%Cl: 90.36%-100%)	36/17 36 (n=17; 47.22%; 95%Cl: 31.98%-62.99%)	36/11 36 (n=11; 30.56%; 95%Cl: 18.01%-46.86%)	36/5 36 (n=5; 13.89%; 95%Cl: 6.08%-28.66%)
> 30 days	Liver (14)	14/14 14 (n=14; 100%; 95%Cl: 78.47%-100%)	14/7 14 (n=7; 50%; 95%Cl: 2.68%-7.32%)	14/4 14 (n=4; 28.57%; 95%Cl: 11.72%-54.65%)	14/1 14 (n=1; 7.14%; 95%Cl: 1.27%-31.47%)
	Blood (27)	27/27 27 (n=27; 100%; 95%Cl: 87.54%-100%)	27/12 27 (n=12; 44.44%; 95%Cl: 27.58%-62.68%)	27/7 27 (n=7; 25.93%; 95%Cl: 13.17%-44.68%)	27/4 27 (n=4; 14.81%; 95%Cl: 5.91%-32.47%)
Season					
Autumn	Liver (16)	16/16 16 (n=16; 100%; 95%Cl: 80.64%-100%)	16/6 16 (n=6; 3.75%; 95%Cl: 18.48%-61.38%)	16/1 16 (n=1; 6.25%; 95%Cl: 1.11%-28.33%)	16/0 16 (n=0; 0%; 95%Cl: 0%-19.36%)
	Blood (37)	37/37 37 (n=37; 100%; 95%Cl: 90.59%-100%)	37/17 37 (n=17; 45.95%; 95%Cl: 31.04%-61.62%)	37/10 37 (n=10; 27.03%; 95%Cl: 1.54%-42.98%)	37/4 37 (n=4; 10.81%; 95%Cl: 4.28%-24.71%)
Winter	Liver (22)	22/22 22 (n=22; 100%; 95%Cl: 85.13%-100%)	22/10 22 (n=10; 45.45%; 95%Cl: 26.92%-65.34%)	22/6 22 (n=6; 27.27%; 95%Cl: 13.15%-48.15%)	22/3 22 (n=3; 13.64%; 95%Cl: 4.75%-33.34%)

	Blood (45)	45/45 45 (n=45; 100%; 95%Cl: 92.13%-100%)	45/22 45 (n=22; 48.89%; 95%Cl: 34.96%-6.3%)	45/14 45 (n=14; 31.11%; 95%Cl: 19.53%-45.66%)	45/7 45 (n=7; 15.56%; 95%Cl: 7.75%-28.79%)
Region					
North	Liver (16)	16/16 16 (n=16; 100%; 95%Cl: 80.64%-100%)	16/5 16 (n=5; 31.25%; 95%Cl: 14.16%-5.56%)	16/2 16 (n=2; 1.25%; 95%Cl: 3.50%-36.02%)	16/0 16 (n=0; 0%; 95%Cl: 0%-19.36%)
	Blood (37)	37/37 37 (n=37; 100%; 95%Cl: 90.59%-100%)	37/15 37 (n=15; 40.54%; 95%Cl: 26.35%-56.51%)	37/9 37 (n=9; 24.32%; 95%Cl: 13.36%-40.11%)	37/2 37 (n=2; 5.41%; 95%Cl: 1.50%-17.71%)
Center	Liver (8)	8/8 8 (n=8; 100%; 95%Cl: 67.56%-100%)	8/2 8 (n=2; 2.50%; 95%Cl: 7.15%-59.07%)	8/0 8 (n=0; 0%; 95%Cl: 0%-32.44%)	8/1 8 (n=1; 1.25%; 95%Cl: 2.24%-47.09%)
	Blood (21)	21/21 21 (n=21; 100%; 95%Cl: 84.54%-100%)	21/5 21 (n=5; 23.81%; 95%Cl: 10.63%-45.09%)	21/4 21 (n=4; 19.05%; 95%Cl: 7.67%-4%)	21/3 21 (n=3; 14.29%; 95%Cl: 4.98%-34.64%)
South	Liver (14)	14/14 14 (n=14; 100%; 95%Cl: 78.47%-100%)	14/9 14 (n=9; 64.29%; 95%Cl: 38.77%-83.66%)	14/5 14 (n=5; 35.71%; 95%Cl: 16.34%-61.23%)	14/2 14 (n=2; 14.29%; 95%Cl: 4.01%-39.95%)
	Blood (24)	24/24 24 (n=24; 100%; 95%Cl: 8.62%-100%)	24/19 24 (n=19; 79.17%; 95%Cl: 9.88%-26.15%)	24/11 11 (n=24; 45.83%; 95%Cl: 27.89%-64.92%)	24/6 24 (n=6; 2.50%; 95%Cl: 1.20%-4.49%)
Total	Liver (38)	38/38 38 (n=38; 100%; 95%Cl: 90.82%-100%)	38/16 38 (n=16; 42.11%; 95%Cl: 27.86%-57.81%)	38/7 7 (n=38; 18.42%; 95%Cl: 9.22%-33.42%)	38/3 38 (n=3; 7.89%; 95%Cl: 2.72%-20.79%)
	Blood (82)	82/82 82 (n=82; 100%; 95%Cl: 95.52%-100%)	82/39 82 (n=39; 47.56%; 95%Cl: 3.71%-58.23%)	82/24 82 (n=24; 29.27%; 95%Cl: 20.53%-38.87%)	82/11 82 (n=11; 13.41%; 95%Cl: 7.66%-22.44%)

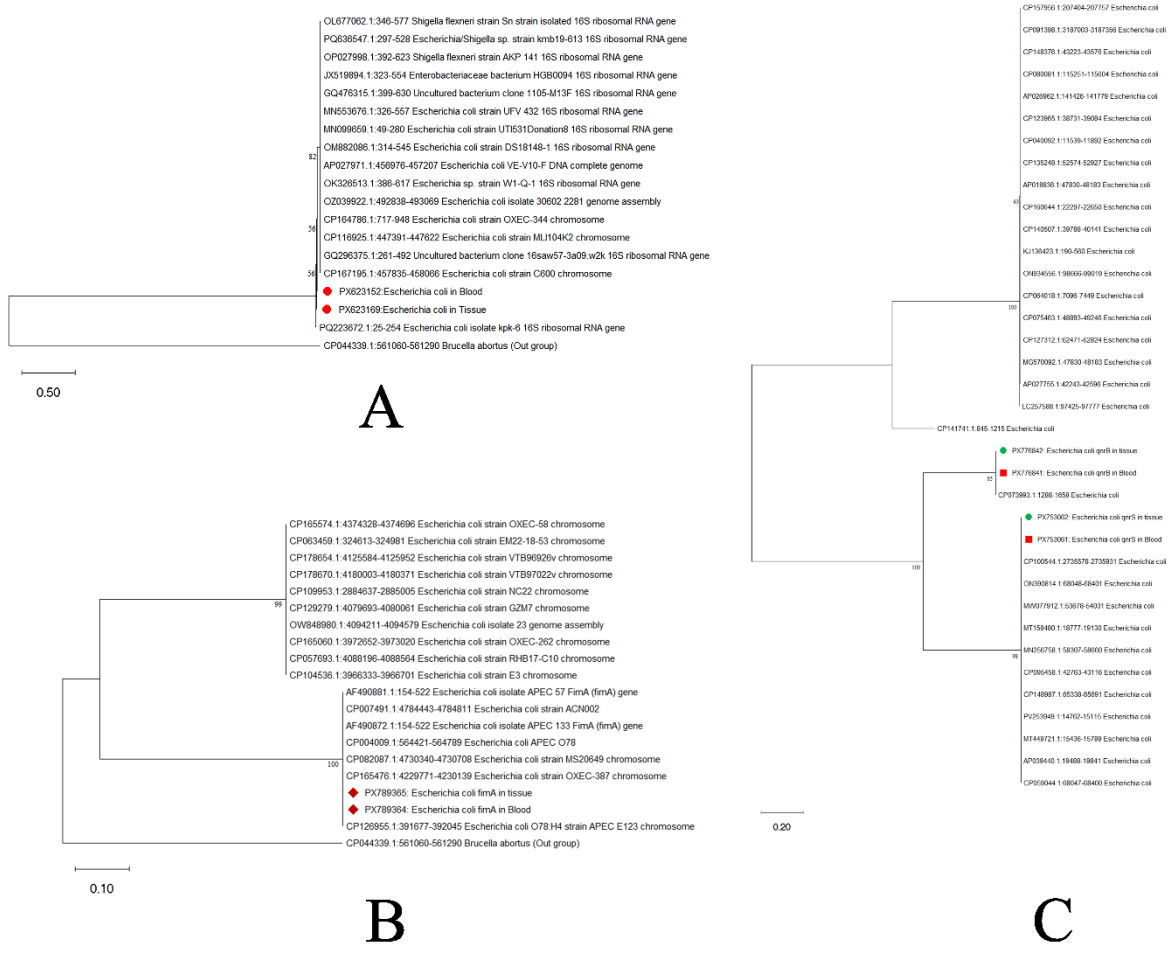

Fig. 3. A: A phylogenetic tree was formulated utilizing partial sequences (286 bp) of the *16S rRNA* gene from *Escherichia coli*, sourced from both our study and the GenBank database. The sequence acquired from our study is prominently marked by a bold circle. **B:** A phylogenetic tree was formulated utilizing partial sequences (321 bp) of the *fimA* genes from *Escherichia coli*, sourced from both our study and the GenBank database. The sequence acquired from our study is prominently marked by a bold circle and square. **C:** A phylogenetic tree was formulated utilizing partial sequences (516 and 469 bp) of the *qnrB and qnrS* genes from *Escherichia coli*, sourced from both our study and the GenBank database. The sequence acquired from our study is prominently marked by a bold diamond. The phylogenetic tree was deduced using the neighbor-joining technique, executed in MEGA 11 software. At each branch point, you can observe bootstrap values that indicate the level of support for the branches. The numerical values positioned above the branches signify the bootstrap support, derived from 1000 replicates.
Red circle: *Escherichia coli* samples isolated from blood of patients - indicating bacterial strains that caused bloodstream infections.
Green circle: *Escherichia coli* samples isolated from tissue - indicating bacterial strains found in different body tissues.

The *qnrS* gene, primarily located on IncN or IncX1 plasmids, induces low-level but stabilizable resistance to fluoroquinolones by inhibiting DNA topoisomerase II and IV enzymes. Its presence at younger ages suggests that this gene can be selected early in the rearing phase, even before clinical symptoms emerge (18). On the other hand, the prevalence of *fimA* which encodes the major

subunit of type 1 fimbriae is also higher at 10 days of age (31.58% in blood isolates versus 16.46% at 30 days), consistent with its role in the initial colonization of the lung and air sac mucosa. The study by Ye et al. (2025) confirmed that early expression of *fimA* coincides with the induction of IL-1β and IL-8 in the lungs, serving as a prerequisite for systemic penetration by APEC. Therefore, the simultaneous presence of *qnrS* and *fimA* in this age group may indicate co-selection for both resistance and virulence a pattern also confirmed by Wang et al. (2024) in 56.3% of APEC isolates in China (19).

The geographic distribution is also noteworthy the prevalence of *qnrB* in the southern region (79.17% in blood isolates) was substantially higher than in the central (23.81%) and northern (40.54%) regions. This marked difference is likely attributable to variations in antimicrobial management practices, stocking density, and environmental conditions (20). Unlike *qnrS*, the *qnrB* gene (particularly the *qnrB*19 subgroup) is more commonly located on small ColE-type plasmids, which persist stably in bacterial populations due to their low fitness cost (8). This characteristic explains why *qnrB* can become locally dominant in a specific region even without ongoing active selective pressure evidence that aligns with the report (21).

Simultaneously, the increased prevalence of *qnrB* and *fimA* during the winter season (48.89% and 31.11%, respectively, in blood isolates compared to 45.95% and 27.03% in autumn) highlights the influence of environmental factors. Reduced ventilation, higher humidity, and ammonia accumulation in colder seasons not only suppress immunity (21) but can also indirectly intensify selective pressure favoring resistant strains (19). Under these conditions, isolates carrying *qnrB* and *fimA* particularly those with strong biofilm-forming ability gain a greater competitive advantage (22). The study by Sasoon et al. (2025) revealed that 96.4% of *E. coli* isolates from Iranian farms exhibit strong biofilm formation, a trait significantly associated with the presence of *qnrB* and *fimA*. Biofilms not only protect bacteria from antibiotics but also provide an ideal environment for horizontal gene transfer via conjugation a phenomenon that heightens the likelihood of co-dissemination of resistance and virulence genes (23).

From a One Health perspective, these findings are indeed alarming. Avian Pathogenic *Escherichia coli* is not only a major pathogen in poultry but is also recognized as a potential genetic reservoir for human Extraintestinal Pathogenic *E. coli*, including pathotypes such as Uropathogenic *E. coli* (24).

The co-localization of *qnrS*, *qnrB* and *fimA* on shared plasmids as reported in studies like Adator et al. (2020) increases the risk of transferring this dangerous combination low-level fluoroquinolone resistance coupled with enhanced adhesion and virulence to humans via the food chain, contaminated water, or direct contact (25). This underscores the interconnected threats of antimicrobial resistance and zoonotic transmission across animal, human, and environmental health domains.

Conclusion

The present findings indicate that *Escherichia coli* populations in broiler farms in West Azerbaijan province are under multidimensional selective pressures including antimicrobial drug use, environmental stressors, and ecological interactions leading to the emergence of strains with coordinated genetic combinations of resistance factors (*qnrS* and *qnrB*) and pathogenicity factors. This genotypic co-occurrence not only demonstrates the co-evolution of resistance pathways (via protection of topoisomerases) and invasion pathways (via primary adhesion to mannosylated receptors), but also serves as an indicator of the increasingly close association between antimicrobial resistance and pathogenicity in avian pathogenic *E. coli*. The presence of qnrS at younger ages, qnrB in specific geographical areas and the increase of fimA in

colder seasons suggest that this selection is shaped by nonlinear interactions between host factors. From a microbial ecology perspective, this pattern may indicate the fixation of resistant-pathogenic strains in a specific ecological niche, where biofilms act as a platform for horizontal transmission of qnr and fim genes. In the One Health context, these strains not only pose a threat to poultry health, but also due to their phylogenetic and genetic similarity to human extraintestinal pathogenic *E. coli* have the potential to systemically transfer transmissible resistance and pathogenicity genes. As a result, current approaches that focus solely on reducing antibiotic use, without integrating biomolecular strategies such as biofilm control, targeted genetic monitoring, and environmental improvements will fail to disrupt the selection cycle of these dual-purpose strains and may simply shift the selection pressure toward more complex resistance mechanisms.

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Conflict of Interest

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Ethical approval

This study did not require formal ethical approval, as all blood and tissue samples were collected exclusively from naturally deceased animals found during routine field surveillance. No live animals were handled, sampled, or subjected to any experimental procedures, and no laboratory animal studies were conducted. Therefore, in accordance with national and institutional guidelines for research involving animal subjects, ethical committee approval or an ethics code was not required for this study.

Artificial Intelligence Statement

The authors confirm that artificial intelligence (AI) tools were used solely for language editing and paraphrasing assistance during the preparation of this manuscript. AI was not involved in any aspect of study design, data collection, analysis, interpretation, or decision-making. The scientific content, methodology, results, and conclusions presented in this work are entirely the authors' own and have been critically reviewed and validated by all co-authors.

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