2026, Scienceline Publication
World's Veterinary Journal
World Vet J, 16(2): 587-596.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2026.wvj56
PII: S232245682600056-16

Prevalence and Molecular Characterization of Antimicrobial Resistance Genes in *Escherichia coli* Isolated from Chicken Meat in Mosul City, Iraq

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ORIGINAL ARTICLE
Received: April 05, 2026
Revised: May 10, 2026
Accepted: June 04, 2026
Published: June 30, 2026

ABSTRACT

Antimicrobial resistance (AMR) in poultry is a growing concern, driven by the widespread use of antibiotics in farming, which can promote the development of resistant bacteria. Resistant pathogens in the food chain can spread AMR and impair the treatment of infectious diseases. The present study aimed to examine the incidence of *Escherichia coli* (*E. coli*) and the presence of AMR genes in chicken meat samples collected from different markets in Mosul City, Iraq. Of 120 samples, 27 isolates (22.5%) tested positive for *E. coli*, identified by chromogenic agar, the VITEK® system, and polymerase chain reaction (PCR) targeting the *uidA* gene. All isolates (27/27; 100%) were confirmed positive for *uidA* detection. The positive isolates were evaluated for five AMR genes, including *dihydrofolate reductase A* (*dfrA*), *β-lactamase* TEM (*bla*TEM), *aminoglycoside acetyltransferase (aac*), *DNA gyrase subunit A* (*gyrA*), and *DNA gyrase subunit B* (*gyrB*). The current findings indicated that 66.7% of the isolates carried at least one resistance gene, and 14.8% carried more than one. The most commonly detected gene was *dfrA* (37.0%), followed by *bla*TEM (29.6%) and *aac* (14.8%). The *gyrA* gene was found in one isolate (3.7%); however, the *gyrB* gene was not detected. Identifying resistance genes causing resistance to commonly used antibiotics, such as trimethoprim, β-lactams, and aminoglycosides, represented a significant public health concern. The current findings can help identify and reduce the spread of AMR across the food supply chain in Iraq.

Keywords: Antimicrobial resistance gene, Chicken meat, *Escherichia coli*, Polymerase chain reaction, Public health

INTRODUCTION

Antimicrobial resistance (AMR) is widely recognized as a major global public health threat. The AMR reduces treatment efficacy, leading to increased morbidity and mortality and substantially increasing healthcare and production costs (WHO, 2023). Antimicrobial medications are extensively used in agriculture, veterinary medicine, and human health care (Marshall and Levy, 2011). Resistant bacteria and their resistance genes can be transferred among humans, animals, food, and the environment (Fusaro et al., 2025). International organizations, such as the World Health Organization, underscored the importance of adopting a One Health approach to effectively address AMR (WHO, 2023). Consequently, efforts should be directed towards monitoring and regulating AMR in humans, animals, and the environment (QJSAR, 2024).

Escherichia coli (*E. coli*) is an important bacterium for studying antibiotic resistance (EFSA and ECDC, 2026). *Escherichia coli* is commonly isolated from the intestines of humans and animals and can cause gastrointestinal infections (EFSA and ECDC, 2026). To assess AMR in livestock, particularly avian species, *E. coli* is frequently examined because it readily acquires and spreads resistance genes via mobile genetic elements such as plasmids and integrons (Marshall and Levy, 2011). *uidA* is a specific gene used to identify *E. coli*; this gene encodes *β-glucuronidase* and is widely recognized for its high specificity for *E. coli* (Bej et al. 1991). The presence of AMR *E. coli* in poultry and poultry products is a significant public health concern, as these bacteria can be transmitted to humans through contaminated food, direct contact with animals, or environmental exposure (Muloi et al., 2018).

The *β-lactamase* TEM (*bla*TEM) gene, which encodes the β-lactamase enzyme, is prevalent in *E. coli* and confers resistance to numerous β-lactam antibiotics (Bush and Bradford, 2016), including penicillin and early cephalosporins such as cephalexin (Katonge and Ally, 2025). The spread of bacteria harboring the *bla*TEM gene compromises the efficacy of β-lactam antibiotics, which are extensively used in human and veterinary medicine (Bush and Bradford, 2016). Therefore, the presence of *bla*TEM-positive *E. coli* in poultry and poultry products indicates a risk of transmitting β-lactam-resistant pathogens from livestock to humans through the food supply (Carattoli, 2013).

Additionally, *dihydrofolate reductase A* (*dfrA*) genes are often identified as a key mechanism by which bacteria acquire resistance to trimethoprim, a medicine frequently combined with sulfonamides to treat bacterial infections

To cite this paper: Al-Mahmood OA, Awed MJM, and Jwher DM (2026). Prevalence and Molecular Characterization of Antimicrobial Resistance Genes in *Escherichia coli* Isolated from Chicken Meat in Mosul City, Iraq. *World Vet. J.*, 16(2): 587-596. DOI: https://dx.doi.org/10.54203/scil.2026.wvj56

(Wang et al., 2025). The *dfrA* gene encodes a modified *dihydrofolate reductase* enzyme that antibiotic agents cannot inhibit (Huovinen, 2001). Plasmids and integrons, which often carry multiple resistance genes, are the most common locations for these genes (Wang et al., 2025). The *dfrA* gene causes resistance to multiple antibiotics in *E. coli*. *Escherichia* coli strains isolated from poultry often harbor *dfrA* genes, reflecting the impact of antimicrobial selection pressure in animal production (Schwarz et al., 2010).

Furthermore, aminoglycoside-modifying enzymes, especially those encoded by *aminoglycoside acetyltransferase* (*aac*) genes, represented a significant mechanism of resistance in *E. coli* (Hemmings et al., 2025). The *aac* enzyme chemically modifies aminoglycoside antibiotics, preventing them from effectively binding to bacterial ribosomes (Hemmings et al., 2025). Consequently, aminoglycoside antibiotics become less effective at eliminating bacteria (Ramirez and Tolmasky, 2010). Additionally, *acc* resistance genes have been commonly identified in *E. coli* isolates derived from chickens, beef, and lamb (Schwarz et al., 2010), indicating that these resistance genes are prevalent in animal production sectors (Schwarz et al., 2010).

Furthermore, mutations in the genes encoding *DNA gyrase subunits A (gyrA)* and *B* (*gyrB*) frequently confer fluoroquinolone resistance in *E. coli* (Jose and Joseph, 2026). The quinolone antibiotics, including levofloxacin and norfloxacin, exert their therapeutic effects by specifically targeting DNA gyrase, a protein complex composed of the *gyrA* and *gyrB* genes. Alterations in DNA gyrase genes subsequently modify the antibiotic's binding site, thereby reducing the medicine's efficacy and the likelihood of fluoroquinolone-induced illness (Hooper and Jacoby, 2016). The proliferation of quinolone-resistant *E. coli* strains in poultry raised significant public health issues, given the importance of quinolones in human medicine (Van Boeckel et al., 2015).

Detecting antibiotic resistance genes in *E. coli* isolates from chicken posed a significant public health concern (EFSA and ECDC, 2026). Poultry products are extensively consumed worldwide, and resistant *E. coli* strains may be transmitted to people via contaminated meat, improper food handling, or environmental contamination during processing and distribution (Islam et al., 2024). Monitoring the occurrence and spread of AMR genes offers vital information. This data can inform antimicrobial stewardship, improve biosecurity and hygiene practices on farms, and strengthen the safety of the food supply chain (WHO, 2025; EFSA and ECDC, 2026). Therefore, the present study aimed to investigate the presence of AMR genes, including *bla*TEM, *dfrA*, *gyrA*, *gyrB*, and *aac*, in *E. coli* isolates collected from chicken meat samples across different markets in Mosul City, Iraq.

MATERIALS AND METHODS

Ethical approval

The present study was approved by the Scientific Committee of the Department of Veterinary Public Health at Mosul University, Iraq (Approval No. 10.9.2025-3). The study was observational and did not employ live or laboratory animals.

Sample collection

A total of 120 chicken meat samples were randomly collected from 15 markets, selected through simple random sampling from available markets across Mosul City, Iraq, between October 2025 and February 2026. Raw and fresh chicken meat samples were aseptically collected using sterile gloves from selected markets. Fresh, packaged chicken samples were purchased and transported to the laboratory under refrigerated conditions (4-6°C) within 12-24 hours. Upon arrival, each package was opened under aseptic conditions, and about 10 mL of meat exudate (drip fluid) was collected using a sterile pipette into sterile containers for microbiological analysis (Martínez-Moreno et al., 2025).

Isolation

Each sample was initially pre-enriched in buffered peptone water (BPW); 1 mL of drip fluid was combined with 9 mL of BPW and incubated aerobically at 37°C for 18 to 22 hours. The enriched broth was streaked onto MacConkey agar (Neogen, UK) using a loop and incubated at 37°C for 24 hours. Lactose-fermenting colonies (pink colonies) suggestive of *E. coli* were selected and sub-cultured for purification. For selective identification, purified isolates were streaked onto chromogenic agar specific for *E. coli* (CHROMagar™ ECC (Himedia, India) and incubated at 37°C for 18-24 hours. Colonies exhibiting typical color changes, from blue to purple, were presumed to be *E. coli*.

Identification using the VITEK system

The VITEK 2 Compact system (bioMérieux, France) was employed to automatically identify presumptive *E. coli* isolates. Pure colonies were subcultured on nutrient agar and incubated at 37°C for 18-22 hours after being isolated from a selective, chromogenic medium. A new colony was suspended in sterile saline and adjusted to a 0.5 McFarland turbidity using a densitometer. The standardized bacterial suspension was inoculated onto VITEK 2 Gram-negative

identification cards. The cards were loaded into the VITEK 2 system, where identification was performed automatically using a series of biochemical reactions and metabolic profiles. The system software interpreted the results, and isolates identified as *E. coli* with high confidence (> 95%) were confirmed.

DNA extraction

Genomic DNA was extracted from confirmed *E. coli* isolates using a commercial bacterial DNA extraction reagent (Addbio, Korea) in accordance with the manufacturer's guidelines. In brief, the lysis buffer was used to resuspend overnight cultures freshly grown on MacConkey agar. The mixture was incubated at 56°C for 20-30 minutes after the addition of Proteinase K, to ensure complete cell dissociation. Ethanol was added to promote DNA binding following lysis, and the lysate was then transferred to a spin column. The column was centrifuged at 10,000 rpm for one minute to facilitate DNA adsorption onto the silica membrane. The column was subsequently cleaned using the provided wash buffers to eliminate contaminants and impurities. Finally, genomic DNA was eluted in 50 µL of elution buffer through centrifugation at 8,000 rpm for one minute. The NanoDrop spectrophotometer (Thermo Scientific, USA) was used to assess DNA integrity and concentration by measuring absorbance at 260/280 nm. The extracted DNA samples were stored at -20°C for polymerase chain reaction (PCR) analysis.

Detection of the *uidA* gene

To confirm the *E. coli* isolates, PCR was employed to amplify the *uidA* gene. Afterward, the isolates were tested for specific AMR genes, including *bla*TEM, *dfrA*, *gyrA*, *gyrB*, and *aac*. A total of 25 µL of PCR reaction mixture was prepared, comprising 12.5 µL of 2x PCR master mix, 1 µL of each primer (forward and reverse), 2 µL of template DNA, and enough nuclease-free water (Al-Mahmood and Fraser, 2023). Table 1 presents the primer sequences, target genes, amplicon sizes, and corresponding references used for the PCR detection of AMR genes in *E. coli* isolates. The PCR was carried out in an Applied Biosystems thermal cycler (USA). The process began with an initial denaturation at 94-95°C for 3-5 minutes, followed by 30-35 cycles of denaturation at 94°C for 30 seconds, annealing of *uidA* at 62°C, *bla*TEM at 59°C, *dfrA* at 61°C, *gyrA* at 58°C, *gyrB* at 55°C, *aac* at 57°C for 30 seconds, and extension at 72°C for 45-60 seconds. A final extension at 72°C lasted 5-7 minutes. The PCR products were then analyzed on a 1.5% agarose gel and compared with a 100 bp DNA ladder.

Table 1. Primer sequences and anticipated amplicon sizes for conventional polymerase chain reaction of *Escherichia coli* isolates

Gene	Primer	Sequence (5′-3′)	Product size (bp)	Reference
uidA	Forward	CCGATCACCTGTGTCAATGT	380	Bower et al. (2005)
	Reverse	GTTACCGCCAACGCGCAATA		
*bla*TEM	Forward	ATCAGCAATAAACCAGC	516	Mabilat and Courvalin (1990)
	Reverse	CCCCGAAGAACGTTTTC		
dfrA	Forward	TGGTAGCTATATCGAAGAATGGAGT	425	Grape et al. (2007)
	Reverse	TATGTTAGAGGCGAAGTCTTGGGTA		
gyrA	Forward	AAATCTGCTCGTGTCGTTGG	349	Ardebili et al. (2015)
	Reverse	GCCATACCTACAGCAATACC		
gyrB	Forward	TACCAACAACATTCCGCAGC	238	De la Fuente et al. (2007)
	Reverse	CGCCGATTTCACCTCAGAAG		
aac	Forward	ATATCGCGATGCATACGCGG	877	Arpin et al. (2003)
	Reverse	GACGGCCTCTAACCGGAAGG		

dfrA: *Dihydrofolate reductase A*, *bla*TEM: *β-lactamase TEM*, *aac*: *Aminoglycoside acetyltransferase*, *gyrA*: *DNA gyrase subunit A*, *gyrB*: *DNA gyrase subunit B*

Statistical analysis

Data obtained from microbiological and molecular analyses were entered into Microsoft Excel and analyzed using John's Macintosh Project **(**JMP) statistical software (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were used to summarize the results. The prevalence of *E. coli* isolates confirmed by the *uidA* gene and AMR genes (*bla*TEM, *dfrA*, *gyrA*, *gyrB*, and *aac*) was calculated as frequencies and percentages.

RESULTS

Escherichia coli isolation

A total of 120 chicken meat samples were examined for the presence of *E. coli*, of which 27 isolates (22.5%) were identified. Initial identification was performed using chromogenic agar, in which presumptive *E. coli* isolates produced blue-to-purple colonies, indicating β-glucuronidase activity typical of *E. coli* (Figure 1). The results of *E. coli* isolates were subsequently confirmed using the VITEK 2 automated identification system (Figure 2).

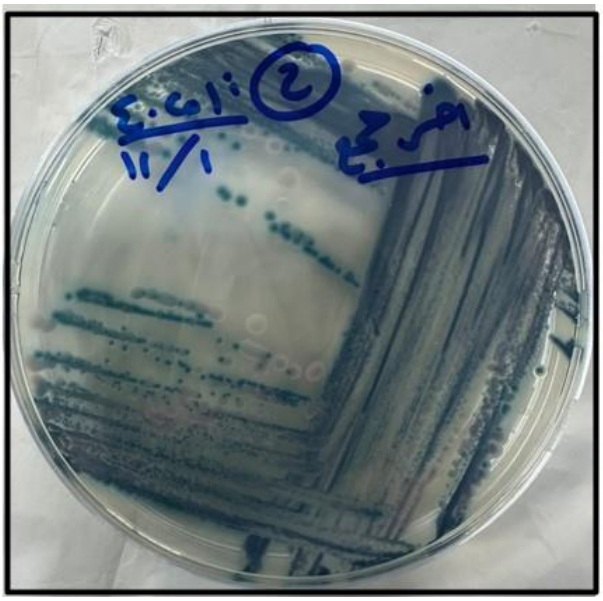

Figure 1. *Escherichia coli* isolates derived from chicken meat samples in Mosul City, Iraq, exhibited blue colonies on chromogenic agar.

bioMérieux Customer: Dr.Radhwan Al-Jammas LAB.
Microbiology Chart Report
Printed February 20, 2026:37:15 PM GMT-06:00

Patient Name: 5, .
Location:
Lab ID: 319
Isolate number:

Organism Quantity:
Selected Organism : Escherichia coli
Source: swab
Collected:

Comments:	

Identification Information	**Analysis Time:**	4.08 hours	**Status:**	Final
Selected Organism	98% Probability	**Escherichia coli**		
	Bionumber:	0405610570526600		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Figure 2. A VITEK 2 automated identification report confirming *Escherichia coli* isolates derived from chicken meat samples in Mosul City, Iraq

Molecular confirmation and antimicrobial resistance gene profiling

All presumptive isolates were confirmed using a molecular method (PCR) to amplify the *uidA* gene, which was unique for *E. coli.* All 27 isolates (100%) were positive for the *uidA* gene, which served as a specific genetic marker for confirming their identity as *E. coli* (Figure 3). The confirmed isolates were subsequently screened for the presence of

five AMR genes (*bla*TEM, *dfrA*, *gyrA*, *gyrB*, and *aac*) using PCR. The present results revealed that all isolates carried resistance genes, and some isolates carried more than one. Among the tested genes, *dfrA* was the most frequently detected (37.0%), followed by *bla*TEM (29.6%) and *aac* (14.8%), whereas *gyrA* was detected in only one isolate (3.7%). The *gyrB* gene was not detected in any of the isolates (Table 2; figures 4-7). The distribution of AMR genes among the 27 *E. coli* isolates revealed that 18 isolates (66.7%) carried at least one resistance gene, indicating a high prevalence of AMR within the studied population, whereas nine isolates (33.3%) did not carry any of the tested resistance genes (Table 3).

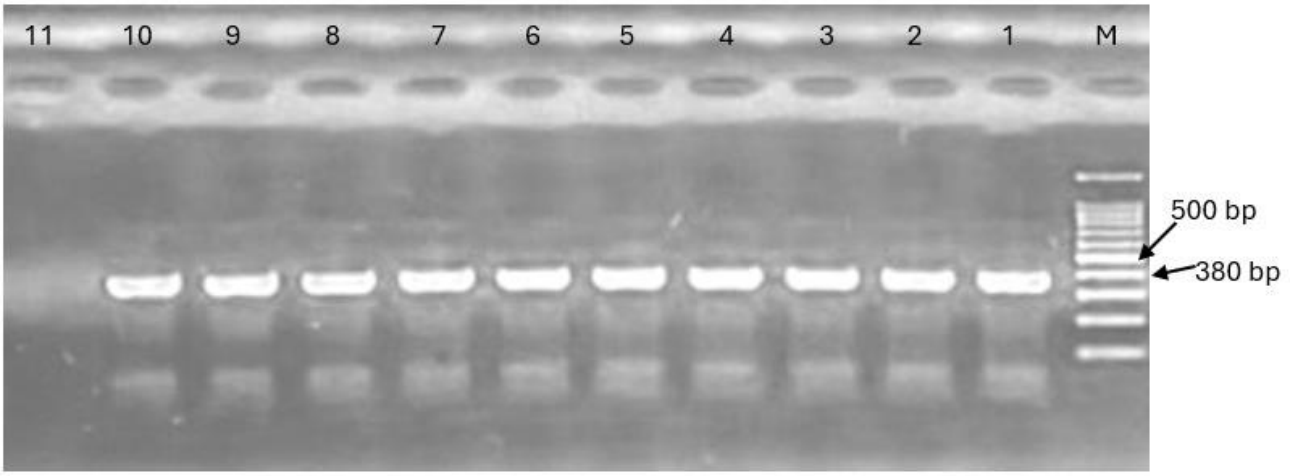

Figure 3. The PCR amplification of the *β-glucuronidase* (*uidA*) gene at a molecular size of 380 bp. Lanes 1-10: Positive samples for *Escherichia coli*, Lane 11: Negative control, Lane M: 100 bp DNA ladder

Table 2. Detection of antimicrobial resistance genes in *Escherichia coli* isolates derived from chicken meat samples in Mosul city, Iraq

Antimicrobial resistance genes	Positive isolates (number)	Percentage (%)
*bla*TEM	8	29.6
dfrA	10	37.0
gyrA	1	3.7
gyrB	0	0
aac	4	14.8

*bla*TEM: *β-lactamase* TEM, *dfrA*: *Dihydrofolate reductase A*, *gyrA*: *DNA gyrase subunit A*, *gyrB*: *DNA gyrase subunit B*, *aac*: *Aminoglycoside acetyltransferase*

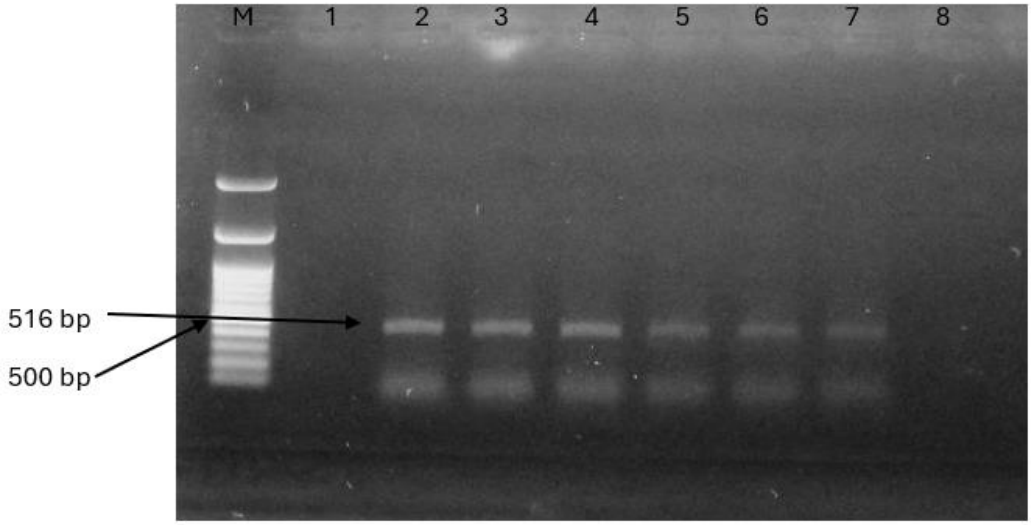

Figure 4. The PCR amplification of the *β-lactamase* TEM (*bla*TEM) gene at a molecular size of 516 bp. Lanes 2-7: Positive samples for *Escherichia coli*, Lane 1: Negative control, Lane 8: Negative sample, Lane M: 100 bp DNA ladder

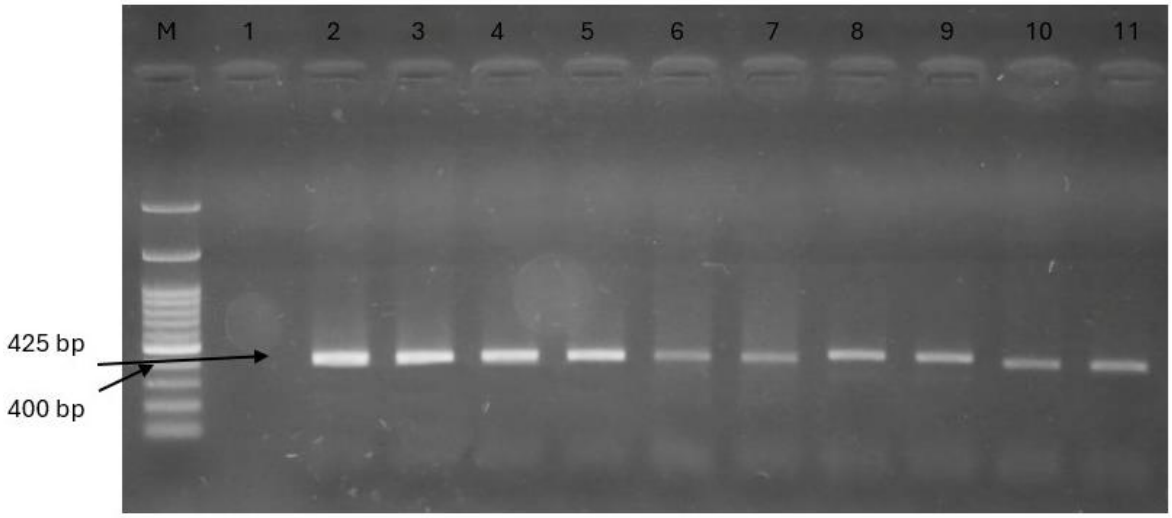

Figure 5. The PCR amplification of the *dihydrofolate reductase A* (*dfrA*) gene at a molecular size of 425 bp. Lanes 2-11: Positive samples for *Escherichia coli*, Lane 1: Negative control, Lane M: 100 bp DNA ladder

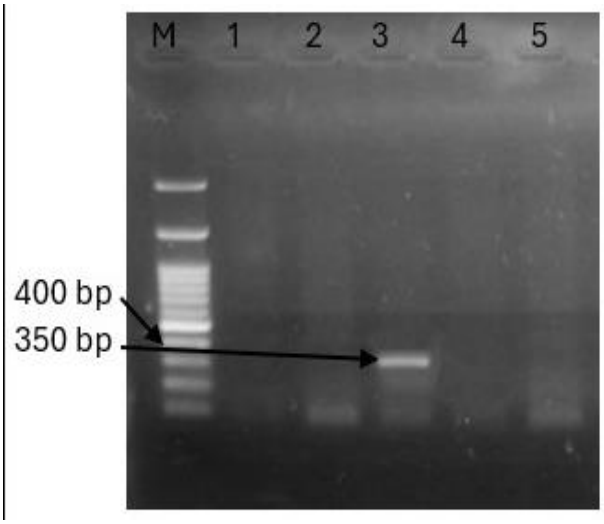

Figure 6. The PCR amplification of the *DNA gyrase subunit A* (*gyrA*) gene at a molecular size of 349 bp. Lane 3: Positive sample for *Escherichia coli*, Lanes 1, 2, 4, 5: Negative samples, Lane M: 100 bp DNA ladder

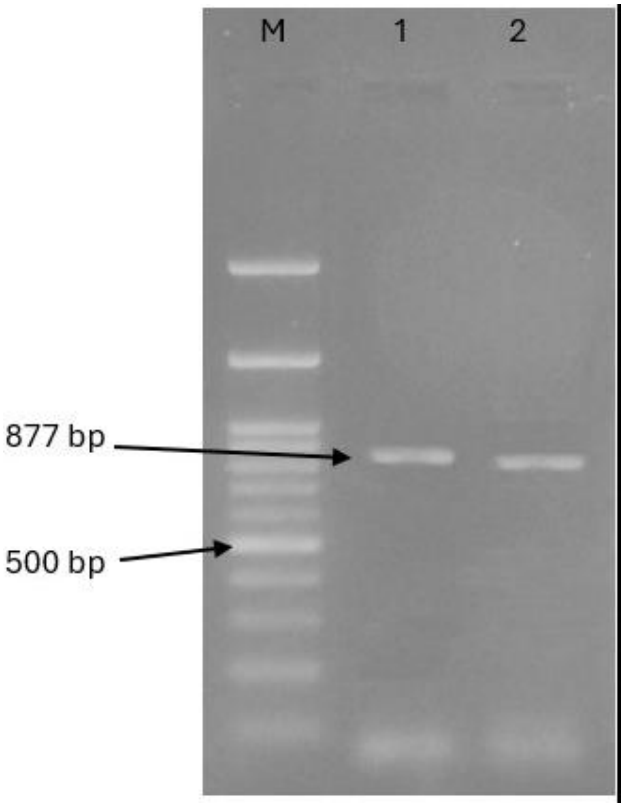

Figure 7. The PCR amplification of the *aminoglycoside acetyltransferase* (*aac*) gene at a molecular size of 877 bp. Lanes 1 and 2: Positive sample for *Escherichia coli*, Lane M: 100 bp DNA ladder

Table 3. The antimicrobial resistance gene distribution in *Escherichia coli* isolates derived from chicken meat samples in Mosul city, Iraq

Category	Number of isolates	Percentage
Isolates with no resistance genes	9	33.3%
Isolates with exactly 1 resistance gene	14	51.9%
Isolates with exactly 2 resistance genes	4	14.8%

DISCUSSION

The widespread presence of *E. coli* in chicken meat and the emergence of AMR genes in its isolates are growing public health challenges globally (Croxen et al., 2013). The use of chromogenic agar, the VITEK® automated identification system, and PCR amplification of the *uidA* gene in the identification process ensured high specificity and sensitivity, which were critical for the surveillance of foodborne pathogens (Fusaro et al., 2025). In the present study, 22.5% of chicken meat samples were contaminated with *E. coli*, consistent with previous studies reporting *E. coli* prevalence in poultry meat ranging from 10% to 48%. In these studies, approximately 100 chicken meat samples were analyzed using microbiological culture, followed by antimicrobial susceptibility testing and PCR to assess resistance patterns (Nhung et al., 2016; Brătfelan et al., 2023).

The detection of AMR genes in 66.7% of *E. coli* isolates was particularly alarming. The present findings reflected global trends of increasing AMR in foodborne pathogens, which have been attributed to the excessive use of antibiotics in poultry production (Van Boeckel et al., 2015; Tang et al., 2017). The detection of *dfrA*, *bla*TEM, and *aac* genes, particularly *dfrA*, aligns with findings from other countries. For instance, in Hong Kong, 180 fresh half-chickens from 29 wet markets were analyzed, and in China, 300 chickens were examined. These studies reported widespread resistance to trimethoprim, β-lactam, and aminoglycoside antibiotics among *E. coli* isolates collected from poultry samples (Hasib et al., 2024; Zhou et al., 2024).

Among the detected genes, *dfrA* had the highest prevalence, at 37.0%, indicating widespread trimethoprim resistance. The current finding was consistent with previous studies demonstrating that trimethoprim resistance genes were commonly found in *E. coli* isolates derived from livestock products, likely due to the widespread use of sulfonamide-trimethoprim combinations in veterinary medicine (Huovinen, 2001; Schwarz et al., 2010). The *dfrA* gene is frequently located on mobile genetic elements such as plasmids and transposons, thereby facilitating their dissemination among bacterial populations (Partridge et al., 2018).

The detection of the *bla*TEM gene in 29.6% of the isolates was substantial, as this gene provided resistance to extended-spectrum β-lactam antibiotics, which are essential in human medicine (Paterson and Bonomo, 2005; Bush and Jacoby, 2010). Faridah et al. (2023) conducted a study in Indonesia involving 115 cloacal swab samples from broiler chickens and found that 38.2% of isolates carried the *bla*TEM gene. This finding indicated that chicken meat could serve as an important reservoir of antibiotic-resistant bacteria, posing potential public health issues. The marginally elevated rate reported in Indonesia may be attributable to the isolates being obtained from live animals (cloacal samples), whereas in the present study, the isolates were derived from chicken meat.

The *aac* gene, associated with aminoglycoside resistance, was detected in 14.8% of isolates, consistent with a previous study reporting a 10% prevalence of aminoglycoside resistance genes in poultry-derived *E. coli* isolates (Brătfelan et al., 2023). The co-occurrence of multiple resistance genes in some isolates (14.8%) further highlighted the potential for multidrug resistance, likely facilitated by plasmid-mediated gene transfer (Carattoli, 2013; Partridge et al., 2018).

The absence of *gyrB* and the low prevalence of *gyrA* (3.7%) among *E. coli* isolates suggested a limited distribution of fluoroquinolone resistance, which could reflect either careful use or limited access to these antimicrobials in Middle Eastern countries (Jacoby, 2005; Chaname Pinedo et al., 2020). However, even a low frequency of fluoroquinolone resistance remains concerning, given the rapid potential for horizontal gene transfer among bacteria (Carattoli, 2013). Consumption of poultry products contaminated with multidrug-resistant *E. coli* can lead to infections that are difficult to treat, increase healthcare costs, and contribute to the global AMR crisis (WHO, 2014; Collignon et al., 2018). The detection of multiple resistance genes in some isolates emphasized the risk of co-selection and the persistence of resistant pathogens in the food chain (Marshall and Levy, 2011; Al-Mahmood, 2023). The current findings indicate that poultry products are a vital reservoir of AMR genes.

CONCLUSION

The current study revealed a high prevalence of AMR among *E. coli* isolates obtained from chicken meat, emphasizing a major concern for food safety and public health in Iraq. The presence of AMR genes in 66.7% of isolates indicated that poultry products could serve as a reservoir for resistant bacteria. The predominance of *dfrA* and *bla*TEM genes suggested widespread resistance to commonly used antimicrobial agents, particularly trimethoprim and β-lactam antibiotics. The presence of multiple resistance genes in some isolates highlighted the potential for multidrug resistance, thereby complicating treatment options and elevating the risk of transmission throughout the food supply chain. Although the prevalence of fluoroquinolone resistance genes was low, their presence still represented a potential threat due to their ability to spread rapidly via mobile genetic elements. The present study had some limitations, including a small sample

size, which may impede the generalization of the results to broader populations. Additionally, the samples were collected exclusively from a single city in Iraq, thereby limiting the ability to demonstrate regional variability. The absence of real-time PCR analysis complicated quantification of antibiotic resistance gene expression, underscoring the need for future studies that employ multi-regional sampling and next-generation sequencing to further assess AMR in *E. coli* isolates derived from poultry samples.

DECLARATIONS

Acknowledgements

The authors expressed their gratitude to all individuals who supported the present study.

Authors' contributions

Omar Ahmad Al-Mahmood contributed to conceptualization, study design, analysis, and writing. Mohammed Jasim Mohammed Awed contributed to the investigation and analysis. Dhyaa Mohammed Jwher contributed to the investigation. All microbiological and molecular analyses were performed by Omar Al-Mahmood. All authors reviewed and approved the final edition of the manuscript.

Availability of data and materials

All datasets underlying the current study are within the manuscript and available upon reasonable request from the corresponding author.

Competing interests

The authors declared no financial or personal conflicts of interest.

Ethical considerations

All authors affirmed that the current study is original, unpublished, not under review elsewhere, and prepared and written without the use of AI tools.

Funding

The present study received no external funding.

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