2025 • Vol. 3, No. 2 • pp. 59-64
Trends in Pharmaceutical Biotechnology (TPB)

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Print ISSN: 3006-0516 Online ISSN: 3006-0354

Analysis of Antibiotic Resistance Gene Expression in Clinical *Escherichia coli* Isolates from Al-Diwaniyah, Iraq

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Access this article online

ORIGINAL A R T I C L E
Received: 12.09.2025 **Revised:** 18.10.2025
Accepted: 18.11.2025
DOI: 10.57238/tpb.2025.153196.1036

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ABSTRACT

Globally, antibiotic resistance in *Escherichia coli* is major health problem. In Iraq, it has become a real issue because many people use antibiotics without proper medical guidance. This resistance comes from bacterial genes that can break the drug or push it out of the cell. Studying these genes at the molecular level is important for understanding how resistance develops and for improving treatment. This work underlines the expression of important resistance genes in clinical E. coli isolates and how gene action is linked to antibiotic resistance. The specimen was obtaining from hospitalized patients and confirm as *E. coli* by standard lab tests. Antibiotic susceptibility analysis was accomplished, as well as three genes—*bla*_{*TEM*}, *aac* (3)-*IV*, and *tetA*—related to β-lactam, aminoglycoside, and tetracycline resistance, were measured by real-time PCR. The results showed β-lactam-resistant isolates had larger *bla*_{*TEM*} expression. Also, *aac* (3)-*IV* and *tetA* were more expressed in strains resistant to aminoglycosides and tetracycline. A very strong link originated between gene overexpression and resistance patterns. These result show that molecular testing is very useful to detect resistance early and to help choose better antibiotics in clinics. This can reduce treatment failure and slow the spread of resistant *E. coli* strains.

Keywords: Antibiotic Resistance, Clinical Isolates, *Escherichia Coli*, Gene Expression, Real-Time PCR, β-Lactamase

1 Introduction

ANTIBIOTIC-resistant bacteria are one of the biggest health problems worldwide. They spread quickly and are a serious threat to modern healthcare. Many certain drugs become less effective, which could origin longer hospital remains, higher costs, and more death rates [1]. Amongst these bacteria, *Escherichia coli* is particularly concerning. It can affect many diseases, like urinary tract infections, bloodstream infections, and some diseases in newborns [2]. In Iraq, antibiotics are often easy to get without a prescription. This weak control has facilitated *E. coli* strains appear and spread fast and becoming resistant [3].

Resistance in *E. coli* usually comes from genes that help bacteria survive antibiotics. These genes could destroy the drug, change its structure, or remove it out of the cell [4–7]. For example, *bla*_{*TEM*} makes an enzyme that destroys β-lactam antibiotics [4]. The *aac (3)-IV* gene modifies aminoglycosides so they do not work, and tetA controls pumps that push tetracycline out of the bacteria [5, 6]. However, Having the genes is not enough. It matters

how much they work. Higher activity usually means stronger resistance and worse treatment outcomes [7].

Quantitative real-time PCR (qRT-PCR) is useful method to measure gene activity. It shows how bacteria respond to antibiotics and can point out strains that are harder to treat [8]. Combining this molecular approach with normal antibiotic tests helps researchers and doctors realize resistance better, track resistant strains, and choose better treatments. This study highlighted the *bla_{TEM}*, *aac (3)-IV*, and *tetA* genes in clinical *E. coli* isolates and compared their activity with antibiotic resistance to guide smarter use of antibiotics.

2 Materials and Methods

2.1 Collection and Processing Samples

A total of 115 clinical samples were gathered. Eighty were from urine and 35 from blood, taken from patients in Al-Diwaniyah General Teaching Hospital between January and August 2025. The samples were sent directly to the microbiology lab and tested by the usual bacteriology methods.

2.2 E. Coli Isolation and Identification

Each specimen was streaked on eosin methylene blue (EMB) agar and then incubated at 37 °C for 24 hours. Colonies showing the typical metallic green sheen were selected for further characterization. Gram staining confirmed Gram-negative bacilli. Biochemical identification was performed using the IMViC tests: isolates were indole and methyl red positive but negative for Voges–Proskauer and citrate utilization. Confirmation was achieved using Triple Sugar Iron (TSI) agar, which revealed an alkaline slant and acid butt without gas or hydrogen sulfide production, consistent with *E. coli*.

2.3 Antibiotic Susceptibility Testing

The antibiotic sensitivity of the verified *E. coli* samples was tested by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (Oxoid, UK), following CLSI 2023 guidelines. The antibiotics included ampicillin (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), ceftazidime (30 µg), imipenem (10 µg), and vancomycin (30 µg). Zones of inhibition were assessed with a digital Vernier caliper, and isolates were classified as susceptible, intermediate, or resistant based on CLSI breakpoints (Table 1).

2.4 RNA Extraction

E. coli isolates were cultured overnight in Luria–Bertani broth at 37 °C with shaking at 180 rpm. Cells were obtained by centrifuging at 5000 × g for 10 minutes, and total RNA was extracted by the RNeasy Mini Kit (Qiagen, Germany) following the manufacturer's instructions. RNA quality and concentration were checked with a NanoDrop™ 2000 spectrophotometer (Thermo Scientific, USA), and only samples with an A260/A280 ratio of 1.8–2.0 were used. Any leftover genomic DNA was discarded using RNase-free DNase I (Thermo Scientific, USA). Extractions were accomplished in triplicate, and negative controls were included to ensure no contamination.

Table 1. Isolates of *E. coli* antimicrobial susceptibility profiles against tested antibiotics.

Antibiotic	Abbreviation	Disk concentration	Susceptibility interpretation
Ampicillin	AMP	10 µg	Susceptible / intermediate / resistant
Gentamicin	GEN	10 µg	Susceptible / intermediate / resistant
Ciprofloxacin	CIP	5 µg	Susceptible / intermediate / resistant
Tetracycline	TET	30 µg	Susceptible / intermediate / resistant
Ceftazidime	CAZ	30 µg	Susceptible / intermediate / resistant
Imipenem	IMP	10 µg	Susceptible / intermediate / resistant
Vancomycin	VAN	30 µg	Susceptible / intermediate / resistant

2.5 cDNA Synthesis

Complementary cDNA was made from 1 µg of RNA applying the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) with random hexamer primers, following the instructions provided by the manufacturer.

2.6 Quantitative Real-Time PCR (qRT-PCR)

The qualified expression of resistance genes (*bla_{TEM}*, *aac (3)-IV*, and *tetA*) was determined by qRT-PCR. Reactions were performed on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, USA) using PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, USA). Specific primers (Figure 1) were obtained from Macrogen Inc. (Seoul, South Korea). Each 20 µL PCR reaction included 10 µL of SYBR Green Master Mix, 0.5 µL of each primer (10 µM), 2 µL of cDNA, and 7 µL of nuclease-free water. The thermal cycling was 95 °C for 5 minutes, then 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Specificity of the PCR products was checked with a melt curve. The 16S rRNA gene served as internal control. All reactions were accomplished in triplicate, and no-pattern controls were involved to ensure specificity.

Fig. 1. Real-Time PCR amplification process and Ct (Cycle Threshold) value determination.

2.7 Data Analysis

The qualified expression of the genes was counted using the 2^−ΔΔCt approach, with desired gene expression normalized to a stable reference gene and compared to susceptible isolates as the control. Fold changes >1 indicate upregulation, while values < 1 indicate downregulation. Statistical comparisons between resistant and susceptible isolates were accomplished using the Mann–Whitney U test in GraphPad Prism v20, with $p < 0.05$ considered statistically significant.

3 Results

3.1 Bacterial Detection

In total, 115 clinical specimens were obtained, consisting of 80 urine and 35 blood samples collected from patients admitted to Al-Diwaniyah General Teaching Hospital between January and August 2025. From these, 10 cultures were confirmed as *E. coli*. On eosin methylene blue (EMB) agar, the isolates produced the typical metallic green sheen (Figure 2A). Microscopic examination showed Gram-negative bacilli. Biochemical testing revealed indole and methyl red positivity, with negative Voges–Proskauer and citrate reactions. Triple Sugar Iron (TSI) agar demonstrated an alkaline slant and acid butt without gas or hydrogen sulfide (Figure 2B), consistent with the profile of *E. coli*.

3.2 Antimicrobial Susceptibility Testing

Out of 115 collected clinical specimens, 10 *E. coli* isolates (E1–E10) were obtained and tested against seven antibiotics. Five isolates (E1–E5) exhibited resistance to at least one drug, while the remaining isolates (E6–E10) were fully susceptible. Ampicillin resistance was most common, detected in all resistant isolates. Gentamicin resistance occurred in three isolates (E1, E2, E5), and tetracycline resistance in three isolates (E1, E3, E4). Notably, isolate E1 was resistant to all three drugs, indicating multidrug resistance. All isolates remained susceptible to ceftazidime, imipenem, and vancomycin (Table 2).

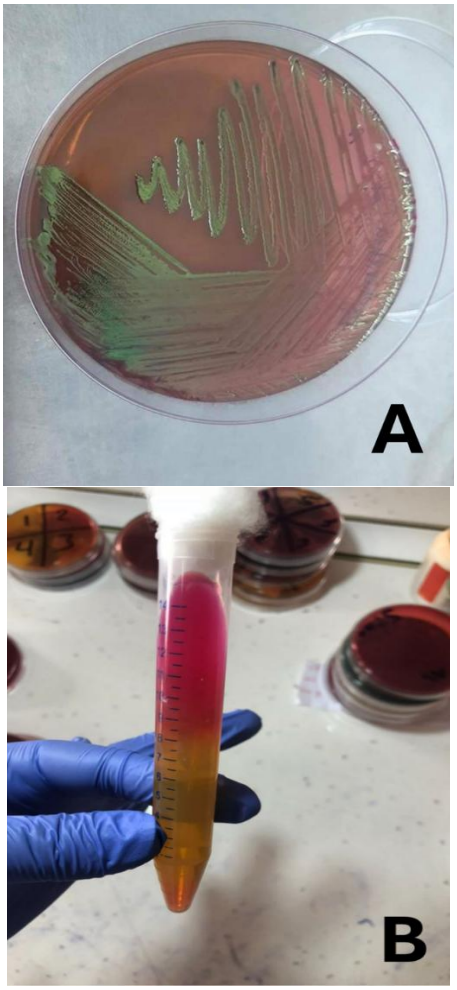

Fig. 2. Identification of *Escherichia coli* (A) colonies on EMB agar showing metallic green sheen (B) TSI agar with an alkaline slant and acid butt, confirming glucose fermentation without gas or H_2S.

Table 2. *E. coli* isolates antimicrobial susceptibility.

Isolate	Ampicillin (AMP)	Gentamicin (GEN)	Tetracycline (TET)	Phenotypic resistance
E1	R	R	R	Resistant
E2	R	R	S	Resistant
E3	R	S	R	Resistant
E4	R	S	S	Resistant
E5	R	R	S	Resistant
E6	S	S	S	Susceptible
E7	S	S	S	Susceptible
E8	S	S	S	Susceptible
E9	S	S	S	Susceptible
E10	S	S	S	Susceptible
R = Resistant, S = Susceptible Interpretation according to CLSI 2023 guidelines.				

3.3 Resistance Gene Expression

qRT-PCR revealed that ampicillin-resistant isolates (E1–E5) overexpressed *bla*_{*TEM*} (3.5–6.2 fold; $p < 0.01$). Gentamicin-resistant isolates (E1, E2, E5) showed elevated *aac (3)-IV* expression (2.8–5.1 fold; $p < 0.05$), and tetracycline-resistant isolates (E1, E3, E4) displayed

increased tetA expression (2.4–4.3 fold; $p < 0.05$). Susceptible isolates (E6–E10) maintained baseline expression. Melt curve and negative controls confirmed specificity and absence of contamination (Figure 3). The important result of relative expression levels of resistance genes in the isolates is presented in Table 3.

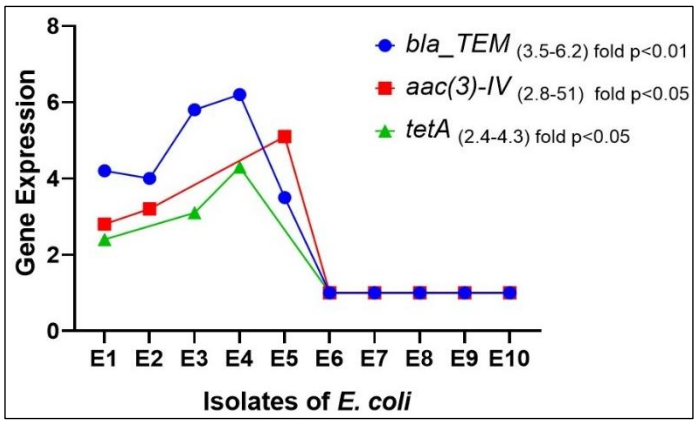

Fig. 3. Differential expression of antibiotic resistance genes in *E. coli* isolates (E1–E5) resistant isolates, (E6–E10) susceptible isolates.

Table 3. Expression profiles of *bla*_{*TEM*}, *aac (3)-IV*, and *tetA* genes in *E. coli*.

Isolate	blaTEM	*aac (3)-IV*	*tetA*
E1	↑	↑	↑
E2	↑	↑	–
E3	↑	–	↑
E4	↑	–	↑
E5	↑	↑	–
E6–E10	Baseline	Baseline	Baseline

3.4 *Correlation Between Phenotype and Genotype*

Phenotypic resistance closely corresponded with gene expression. All ampicillin-resistant isolates overexpressed *bla*_{*TEM*}, gentamicin resistance correlated with *aac (3)-IV*, and tetracycline resistance aligned with tetA upregulation. The multidrug-resistant isolate (E1) demonstrated simultaneous overexpression of all three genes, reflecting its broad resistance profile. These results highlight the genetic basis of antibiotic resistance in the tested *E. coli* isolates and underscore the utility of molecular diagnostics for resistance monitoring as shown in Figure 4.

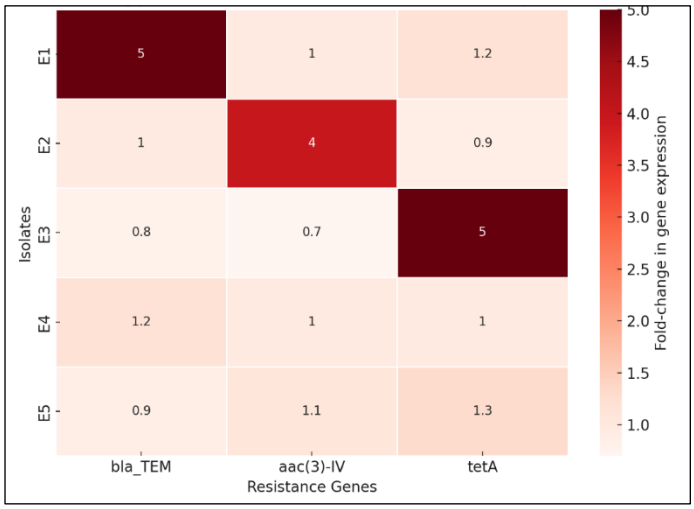

Fig. 4. Heatmap showing fold-change in expression of antibiotic resistance genes across *E. coli* isolates. Resistance genes (*bla*_{*TEM*}, *aac (3)-IV*, *tetA*). E1 shows simultaneous overexpression of all three genes, reflecting its multidrug-resistant phenotype.

4 Discussion

This study instructed a clear relationship between phenotypic resistance and overexpression of resistance-associated genes in the 10 mentioned clinical *E. coli* isolates obtained from 115 specimens. Among the five ampicillin-resistant isolates (E1–E5), *bla*_{*TEM*} expression was 3.5- to 6.2-fold higher than in susceptible isolates, indicating that transcriptional upregulation contributes significantly to β-lactam resistance. This gene encodes a β-lactamase enzyme that hydrolyzes the β-lactam ring of penicillin, inactivating the drug before it can inhibit penicillin-binding proteins in the bacterial cell wall [9].

Gentamicin-resistant isolates (E1, E2, E5) exhibited 2.8- to 5.1-fold increased expression of *aac (3)-IV*, which encodes an aminoglycoside 3-N-acetyltransferase. This enzyme chemically modifies aminoglycosides, preventing them from binding to the 30S ribosomal subunit and disrupting protein synthesis [10]. Notably, isolate E3 displayed the highest fold change, which may reflect selective pressure from aminoglycoside use or clonal expansion of a highly resistant strain [11].

Tetracycline-resistant isolates (E1, E3, E4) showed 2.4- to 4.3-fold upregulation of *tetA*, encoding a membrane-associated efflux pump that actively expels tetracycline from the cell. Even though the fold increases were lower than those observed for *bla*_{*TEM*}, modest upregulation could decrease intracellular drug concentrations enough to allow bacterial survival under antibiotic stress [12, 13].

In general, the observed forms demonstrate that resistance mechanisms in E. coli due to enzymatic degradation, chemical modification, as well as active efflux that relate closely to phenotypic resistance profiles [14, 15]. The multidrug-resistant isolate (E1), which overexpressed *bla*_{*TEM*}, *aac (3)-IV*, and *tetA* simultaneously,

illustrates the cumulative effect of multiple resistance strategies.

These results demonstrate that observing gene expression could help predict if bacteria will resist antibiotics [16]. In places like Iraq, where people can get antibiotics easily and hospitals have limited testing, using methods like qRT-PCR in routine checks could help find resistant bacteria earlier, choose the right treatment, and improve antibiotic use [17, 18]. Other studies support this work as found about resistant genes in *E. coli*. For instance, a 2023 work by Gálvez-Benítez et al. found more copies of the *bla_TEM* gene in *E. coli* that cannot respond to piperacillin-tazobactam, caused by gene duplications and plasmid increases [19]. As well, a 2025 study by Elmorsy et al. showed that tetA was one of the extremely widespread resistance genes in *E. coli* from poultry, showing its role in tetracycline resistance [20]. Thus, these studies show gene expression is important to understand and limit antibiotic resistance.

5 Conclusion

These findings demonstrate a clear association between the overexpression of *bla*_{*TEM*}, *aac (3)-IV*, and the observed resistance to ampicillin, gentamicin, and tetracycline, respectively, in the 10 clinical *E. coli* isolates obtained from 115 specimens. The multidrug-resistant isolate (E1), which showed upregulation of all three genes at the same time, shows how serious the problem is when one strain carries many resistance ways. These results point out that checking resistance genes with tools like qRT-PCR is really helpful. It can help find resistant bacteria early, pick the right antibiotics, and reduce the spread of resistant E. coli in hospitals.

As future work, laboratories could try using molecular tests like qRT-PCR. This technique could give immediate and accurate results for resistance genes. Still, normal phenotypic tests should be used too. More studies with further isolates are needed to show how these genes are spread. Studying why some strains have very high gene expression, maybe from promoter changes or extra plasmid copies, can help explain how resistance develops. Also, following how antibiotic-stewardship programs change gene expression in hospital pathogens might aid improve treatment choices and slow multidrug resistance.

Acknowledgment: The authors thank the University of Al-Qadisiya, Iraq, especially the Department of Pathological Analyses and the Department of Environmental Research and Pollution Prevention, College of Science, for lab facilities and technical support. We also thank the Polytechnic College, Al-Qadisiyah, Al-Furat Al-Awsat Technical University, Iraq, for help with the experiments, and Warwick University, UK, for their guidance and cooperation. We are grateful to Diwaniyah Hospital for their help and for providing clinical samples needed for this work.

Conflict of Interest: The author declares no conflict of interest.

Financing: The study was performed without external funding.

Ethical consideration: The study was approved by Babylon University, Hillah, Iraq.

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How to cite this article

Obaid B.H.; Al-Ganahi S.A.; Farzand R.; Kadhim B.A.; Ali R.J.; Gene Expression Analysis of Antibiotic Resistance-Associated Genes in Clinically Isolated *Escherichia Coli* in Al-Diwaniyah, Iraq. Trends in Pharmaceutical Biotechnology (TPB). 2025;3(2):59-64. doi: 10.57238/tpb.2025.153196.1036