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Original Research Paper

Gene profile of *acrA* and *acrB* efflux pump genes in multidrug-resistant clinical *E. coli* isolates

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Background & Objective: Antibiotic resistance in *Escherichia coli* is a major global challenge. This study aimed to evaluate the prevalence of two key genes, *acrA* and *acrB*, and investigate their association with antibiotic resistance patterns in various bacterial isolates. **Methods**: A total of 71 *E. coli* isolates were collected and subjected to antibiotic susceptibility testing. Subsequently, PCR (Polymerase Chain Reaction) was performed to detect the presence of the *acrA* and *acrB* genes. Statistical analysis was then conducted to determine the correlation between these genes and the observed resistance phenotypes. **Results**: The statistical analysis revealed a significant correlation between the *acrA* gene and three specific antibiotics (Aztreonam, Azithromycin, and Amikacin). Regarding the *acrB* gene, a significant statistical association was found with only two antibiotics: Colistin and Aztreonam. No significant correlation was observed with the remaining tested antibiotics. **Conclusion**: The findings conclude that the *AcrA*B efflux pump system is directly involved in mediating resistance to Colistin, Aztreonam, Amikacin, and Azithromycin. Resistance to other antibiotic classes is likely attributed to alternative bacterial defense mechanisms, such as the production of beta-lactamases or alterations in membrane permeability (Porins).

Keywords: *Escherichia coli*; Multidrug resistance; Efflux pumps; *acrA* and *acrB* genes; Clinical isolates**.**

1. Introduction

Escherichia coli is one of the most frequent multidrug-resistant pathogens. It possesses efflux pumps, which are considered a vital mechanism for this bacterium's resistance [1]. resistance-nodulation-division (RND) family includes the *AcrA* and *AcrB* components [encoded by *acrA* and *acrB* genes] [2]. Multiple Antibiotic Resistance (MAR) in bacteria is caused by active efflux [3].In *E. coli*, overproduction of the *AcrA*B efflux pump confers multiple antibiotic resistance (MAR) to various compounds [4, 5]. In *E. coli*, the *acrA* and *acrB* genes can be regulated by two proteins: the MarA protein and the SoxS protein. These genetic loci [of both genes] are members of the *mar* and *sox* regulons [6]. To prevent antibiotic resistance, the antibiotic prescription must be taken correctly by considering three factors: time, administration manner, and dose [7, 8]. Bacterial multidrug efflux systems are a widespread and common mechanism employed by bacteria for resistance[9]. These systems are capable of extruding various types of structurally diverse antibiotics, as well as certain metabolic products. Furthermore, in Gram-negative bacteria, these efflux pumps are physiologically essential for processes such as biofilm formation[9]. In Gram-negative bacteria, the most clinically significant efflux pumps are members of the RND family, as they are directly associated with multidrug resistance (MDR)[9]. "These pumps exist as a tripartite system, spanning across both the inner and outer membranes[9, 10]. Due to the high level of homology at both the gene and protein levels in multidrug resistance systems, the *AcrA*B-TolC system was adopted as the prototype for this class of pumps and has been extensively studied [11]. The increased expression (overexpression) of *acrA* and *acrB* genes leads to a higher efflux of substances,

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which directly enhances antibiotic resistance in *E. coli* [5]. Mutations within local repressor genes can lead to the overexpression of efflux pumps [12-14]. In *E. coli*, the overexpression of efflux pumps can result from the activation of a regulon [a group of genes] regulated by two key proteins: the MarA protein and the SoxS protein [15, 16]

In the current study, bacterial isolates were collected and identified using the VITEK system from several laboratories in Najaf, Iraq, including the Public Health Laboratory, Al-Asimah Laboratory, Al-Safeer Laboratory, and Sabah Al-Fatlawi Laboratory. The study was conducted from September 2025 to December 2025. Antimicrobial susceptibility testing was performed, and the results were correlated with the presence of efflux pump resistance genes, specifically *acrA* and *acrB*. This was done to investigate the relationship between the tested antibiotics and these specific genes within *Escherichia coli* isolates.

2. Methodology

Sample Collection and Study Design

This cross-sectional study was conducted to investigate the association between antibiotic resistance in Escherichia coli and two efflux pump genes, namely acrA and acrB. A total of 71 isolates were collected from various clinical specimens, including High Vaginal Swabs (HVS), Seminal Fluid Analysis (S.F.A), Urinary Tract Infections (UTI), and Pleural Fluid. These samples were obtained between September 2025 and December 2025. The isolates were sourced from the Public Health Laboratory as well as several private laboratories in Al-Najaf. Furthermore, ethical approval for this study was granted by the Ethics Committee of the Al-Najaf Health Directorate, Iraq.

Culture and Identification of Bacteria

These samples were processed in the Public Health Laboratory and various private laboratories. Primary cultivation was performed using selective and differential media, including MacConkey agar, Blood agar, and Nutrient agar (Thermo Fisher Scientific, USAP). Bacterial identification was subsequently carried out through a series of biochemical tests, such as catalase, urease, H2S production, and citrate utilization tests. All cultivation procedures and biochemical assays were conducted in strict accordance with established microbiological manuals [17, 18].

Antibiotic Sensitivity Test

We performed the antibiotic sensitivity tests according to the microbiology laboratory standards [19]. The activity of several antibiotics was assessed on Mueller-Hinton agar using the disc diffusion method. We tested various antibiotics, including cefotaxime, amoxicillin, meropenem, ceftriaxone, nitrofurantoin, azithromycin, tobramycin, colistin, trimethoprim, tetracycline, imipenem, fosfomycin, gentamicin, penicillin, doxycycline, aztreonam, and amikacin. which are available and commonly used routinely in our hospitals. The concentration of all antibiotics disks are 30 µg and 10 µg.

Molecular Detection of acrA gene

isolated the bacterial DNA using a DNA extraction kit for Gram-negative bacteria from Bio-Solar kit by following the manual leaflet inside the kit. DNA amplification by PCR was performed using specific primers for the acrA and acrB genes and Taq-polymerase master mix (Promega, USA). The forward and reverse primer sequences are 5'- ggtcgttctgatgctctca -3' and 5'- ggcttgctggttattatcag -3' respectively for acrA gene [20]. The thermal cycling of the PCR of three steps;. initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension step was performed at 72°C for 5 minutes.

Molecular Detection of acrB gene

The forward and reverse primer sequences are 5'- cgtctaacagtgactccacgg -3' and 5'- ttcaatcagacctttaccttc -3' respectively for acrB gene which are synthesized by Macrogen, South Korea [20]. initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 45 seconds. A final extension step was performed at 72°C for 10minutes [21]. Electrophoresis for the PCR products was performed

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using 1% agarose gel containing ethidium bromide (Thermo Fisher USA) and then visualized by UV illuminator.

3. Results and discussion

Distribution of Escherichia coli Isolates Based on Clinical Sources and Detection of arcA and arcB Genes

A total of 71 *E. coli* isolates were analyzed in this study. The distribution according to clinical sources showed that 50 (70.4%) isolates were obtained from Urinary Tract Infections (UTI), followed by 11 (15.5%) from High Vaginal Swabs (HVS). Additionally, 9 (12.7%) isolates were recovered from Seminal Fluid Analysis (SFA) samples, and 1 (1.4%) isolate was from Pleural Fluid (Table 1). The distribution of the studied genes showed that the arcA gene was detected in 39 (54.9%) of the isolates, while 32 (45.1%) were negative. In contrast, the arcB gene was detected in 9 (12.7%) isolates, whereas 62 (87.3%) were negative (Table 2).

Age and Gender Distribution and Antimicrobial Resistance Patterns of E. coli Isolates

The findings indicate that E. coli isolates were more frequently detected within the productive age group for both males and females. The mean age of female patients was slightly higher than that of males (37 years compared to 34 years). This close age range suggests that both genders show a similar susceptibility to E. coli infections in this clinical sample (Figure 1).

Regarding antimicrobial resistance, the isolates showed a very high resistance rate to Cefotaxime (100%) and a high resistance rate to Ceftriaxone (88.7%). These findings may indicate the possible presence of Extended-Spectrum Beta-Lactamase (ESBL) producing strains among the isolates. It is well documented that Gram-negative bacteria such as E. coli often exhibit higher resistance levels to antibiotics compared with many other bacterial groups.

While previous studies reported that Imipenem and Amikacin were among the most effective antibiotics against uropathogenic bacteria, the present findings demonstrate that Meropenem and Colistin showed high therapeutic effectiveness against the studied multidrug-resistant (MDR) E. coli isolates (Table 3). These observations highlight the continued importance of carbapenems and polymyxins as effective treatment options for infections caused by resistant E. coli strains.

Table1: Distribution of Isolates According to Clinical Source

Infection Category of Patient	Count
UTI	50
Pleural Fluid	1
HVS	11
S.F.A	9

Table 2: Frequency and percentage of porin genes (arcA and arcB) among the studied *E. coli* isolates (N = 71).

Gene	**Positive [No.]**	**Positive [%]**	**Negative [No.]**	**Negative [%]**
arcA	39	54.9%	32	45.1%
arcB	9	12.7%	62	87.3%

Table 3: Antibiotic susceptibility pattern among isolates (n = 71)

Antibiotic	**Resistant [%]**	**Intermediate [%]**	**Sensitive [%]**
Cefotaxime	71 [100%]	0 [0%]	0 [0%]
Axamoxicillin	64 [90.1%]	2 [2.8%]	5 [7.0%]
Meropenem	7 [9.9%]	1 [1.4%]	63 [88.7%]
Ceftriaxone	63 [88.7%]	7 [9.9%]	1 [1.4%]
Nitrofurantoin	55 [77.5%]	15 [21.1%]	1 [1.4%]
Azithromycin	46 [64.8%]	10 [14.1%]	15 [21.1%]
Tobramycin	19 [26.8%]	41 [57.7%]	11 [15.5%]

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Colistin	1 [1.4%]	0 [0%]	70 [98.6%]
Trimethoprim	38 [53.5%]	2 [2.8%]	31 [43.7%]
Tetracycline	41 [57.7%]	6 [8.5%]	24 [33.8%]
Imipenem	27 [38.0%]	41 [57.7%]	3 [4.2%]
Fosfomycin	66 [93.0%]	5 [7.0%]	0 [0%]
Gentamicin	15 [21.1%]	21 [29.6%]	35 [49.3%]
Penicillin	64 [90.1%]	7 [9.9%]	0 [0%]
Doxycycline	27 [38.0%]	13 [18.3%]	31 [43.7%]
Aztreonam	40 [56.3%]	8 [11.3%]	23 [32.4%]
Amikacin		41 [57.7%]	28 [39.4%] 2 [2.8%]

acrA Gene PCR Amplification

The molecular detection of *acrA* Gene was performed on 71 *E. coli* isolates. The PCR amplification results revealed that 39 (54.9%) isolates were positive for the *acrA* Gene (Figure 2). For the *acrA* gene, 39 out of 71 *E. coli* isolates were positive (54.9%). The mean percentage of the positive isolates for the acrA gene was (56.9%). Resistance predominated among isolates harboring the *acrA* gene, and among gene-positive isolates, the majority were resistant to the tested antibiotics (Table 4).

Table 4: Antimicrobial resistance profile among *acrA* -positive *Escherichia coli* isolates (n = 39/71), expressed as number and percentage (n/N%).

Antibiotic	**Resistant n/N [%]**	**Sensitive n/N [%]**
Cefotaxime	39/39 [100.0%]	0/39 [0.0%]
Amoxicillin	36/39 [92.3%]	3/39 [7.7%]
Meropenem	4/39 [10.3%]	35/39 [89.7%]
Ceftriaxone	36/39 [92.3%]	3/39 [7.7%]
Nitrofurantoin	31/39 [79.5%]	8/39 [20.5%]
Azithromycin	21/39 [53.8%]	18/39 [46.2%]
Tobramycin	11/39 [28.2%]	28/39 [71.8%]
Colistin	1/39 [2.6%]	38/39 [97.4%]
Trimethoprim	20/39 [51.3%]	19/39 [48.7%]
Tetracycline	24/39 [61.5%]	15/39 [38.5%]
Imipenem	15/39 [38.5%]	24/39 [61.5%]
Fosfomycin	36/39 [92.3%]	3/39 [7.7%]
Gentamicin	8/39 [20.5%]	31/39 [79.5%]
Penicillin	35/39 [89.7%]	4/39 [10.3%]
Doxycycline	15/39 [38.5%]	24/39 [61.5%]
Aztreonam	27/39 [69.2%]	12/39 [30.8%]
Amikacin	18/39 [46.2%]	21/39 [53.8%]
Mean	56.9%	

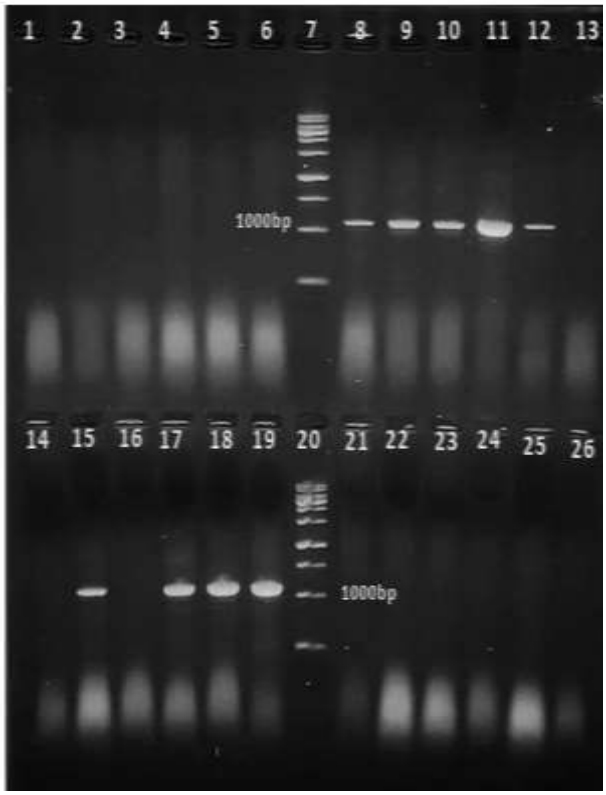

Fig.1. Agarose gel electrophoresis for PCR products of *acrA* Gene amplification. Lanes 8,9,10,11,12,15,17,18 ,and 19 are showing positive PCR amplifications that are 1078bp in size. Lanes 1,2,3,4,5,6,13,14,16,21,22,23,24,25, and 26 are showing

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negative PCR amplifications. Lanes 7 and 20 are a standard DNA ladder.

acrB Gene PCR Amplification

The molecular detection of *acrB* Gene was performed on 71 *E. coli* isolates. The PCR amplification results revealed that that 9 (12.7 %) isolates were positive for the *acrB* Gene (Figure 2). //For the *acrB* gene, 9 out of 71 isolates were positive (12.7%). Similarly, resistance predominated among isolates carrying the *acrB* gene, with most gene-positive isolates showing resistance to the tested antibiotics. The mean percentage of antibiotic resistance among the acrB gene-positive isolates was (62.7%) (Table5).

Table 5: Antimicrobial resistance profile among *acrB* - positive *Escherichia coli* isolates (n = 9/71), expressed as number and percentage (n/N%)

Antibiotic	Resistant n/N [%]	Sensitive n/N [%]
Cefotaxime	9/9 [100.0%]	0/9 [0.0%]
Amoxicillin	9/9 [100.0%]	0/9 [0.0%]
Meropenem	1/9 [11.1%]	8/9 [88.9%]
Ceftriaxone	9/9 [100.0%]	0/9 [0.0%]
Nitrofurantoin	7/9 [77.8%]	2/9 [22.2%]
Azithromycin	4/9 [44.4%]	5/9 [55.6%]
Tobramycin	3/9 [33.3%]	6/9 [66.7%]
Colistin	1/9 [11.1%]	8/9 [88.9%]
Trimethoprim	5/9 [55.6%]	4/9 [44.4%]
Tetracycline	7/9 [77.8%]	2/9 [22.2%]
Imipenem	2/9 [22.2%]	7/9 [77.8%]
Fosfomycin	9/9 [100.0%]	0/9 [0.0%]
Gentamicin	3/9 [33.3%]	6/9 [66.7%]
Penicillin	9/9 [100.0%]	0/9 [0.0%]
Doxycycline	5/9 [55.6%]	4/9 [44.4%]
Aztreonam	8/9 [88.9%]	1/9 [11.1%]
Amikacin	5/9 [55.6%]	4/9 [44.4%]
Mean	62.7%	

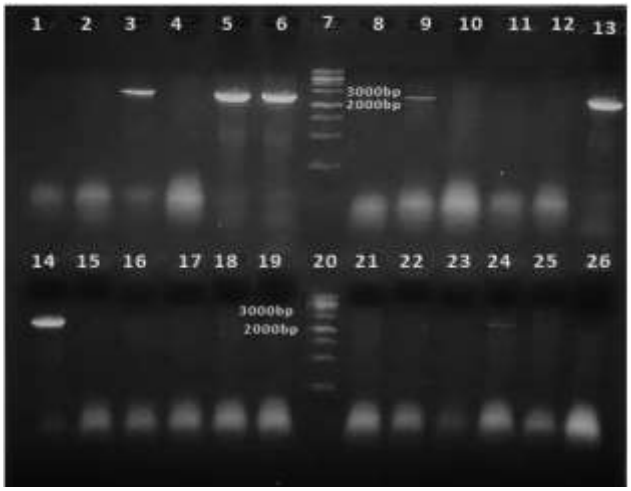

Fig 2: Agarose gel electrophoresis for PCR products of *acrB* Gene amplification. Lanes 3,5,6,9,13,and 14 are showing positive PCR amplifications that are 2730 bp in size. Lanes 1,2,4, 8,10,11,12,15,16,17,18,19,21,22,23,24,25,and 26 are showing negative PCR amplifications. Lane 7and 20 are a standard DNA ladder.

In the current study, a total of 71 *Escherichia coli* isolates were recovered from various clinical sources. The distribution showed that Urinary Tract Infections (UTIs) were the most frequent source, accounting for 50 samples, followed by High Vaginal Swabs (HVS) with 11 samples, 9 isolates from Seminal Fluid Analysis (SFA), and 1 isolate from Pleural Fluid. Consistent with global clinical data, *Escherichia coli* remains the most frequently encountered uropathogen in urinary tract infections [22-24].

Locally, our results mirror previous investigations in Al-Najaf, where *E. coli* was identified as the leading cause of UTIs, representing 48% of 1148 positive cases. This reinforces the status of this bacterium as the primary agent responsible for the majority of UTI infections in the local community [18].

The phenotypic resistance profiles in this study demonstrated a critical level of multi-drug resistance. Notably, we observed a 100% resistance rate to Cefotaxime. This phenomenon can be attributed to the role of polyamines and membrane permeability. According to previous studies, these polyamines function by decreasing the permeability of the outer membrane in *Escherichia coli*. Even in the absence of efflux pumps, polyamines can effectively block the

porins located in the outer membrane, thereby preventing the entry of antibiotics into the bacterial cell [25-27].

Furthermore, the resistance observed against Amoxicillin in these isolates can be linked to the physiological role of efflux pumps in transporting polyamines. Since Amoxicillin shares similar physiochemical characteristics with endogenous polycation substrates (such as polyamines), the pump identifies the antibiotic as a natural substrate. Consequently, the extrusion of Amoxicillin occurs as a by-product of the pump's normal physiological function, leading to the development of resistance in these isolates [28-30].

The molecular component of this work focused on the RND-family efflux system. In our study, the *acrA* gene was detected in 55% of the isolates. This finding aligns with the fact that efflux pumps are considered ancient genetic elements, conserved in approximately 80% of *Escherichia coli* strains. This suggests that the efflux pump system is not merely a mechanism for antibiotic resistance, but is also fundamentally involved in essential physiological functions [31, 32].

Regarding the *acrB* gene, the isolates positive for this determinant demonstrated high resistance to Colistin. This can be explained by the physiological role of the *acrB* pump in maintaining membrane lipid homeostasis. While Colistin functions by targeting and disrupting the bacterial outer membrane, the presence of the *acrB* gene allows *E. coli* to repair the membrane by extruding damaged lipids or modulating lipid composition. Consequently, *E. coli* isolates harboring this gene exhibit enhanced survival and superior resistance against the membrane-disrupting effects of Colistin [2, 33, 34].

Finally, the lower prevalence of the *acrB* gene in *E. coli* isolates compared to the *acrA* gene can be attributed to functional redundancy. As other genetic systems may perform the same physiological roles on its behalf, the bacteria maintains its survival capacity even with a lower frequency of this specific gene [35].

Conclusion

Using antibiotics without testing their activity against pathogens would most likely lead to improper use of treatment that triggers resistance by pathogenic bacteria. The results of the present study revealed increased rates of resistance. Molecular screening for the *acrA* and *acrB* in *E. coli* suggests a high incidence of genetic alteration and developing drug resistance in bacteria. Occasional screening and assessment of antibiotic resistance is highly recommended to evaluate the activity of empirical drugs prescribed against the etiological agents.

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Ethical Consideration

The study was accepted by the University of Kufa's College of Science Ethics Committee, in December 2025. Ethical approval was obtained, and informed consent was secured from all participating patients before specimen collection. Duplicate samples from the same patient were excluded to avoid redundancy in data analysis.

Conflict of Interest

The author declared no conflict of interest.

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