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Original Article

Prevalence of Beta-Lactamase resistance genes in *Escherichia coli* isolated from individuals with renal dysfunction

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Abstract

Background & Objectives: The emergence of antibiotic resistance in *Escherichia coli* (*E. coli*), particularly among extended-spectrum beta-lactamase (ESBLs) producing strains, represents a growing global health concern. Patients with renal failure are especially vulnerable to recurrent hospitalizations and invasive interventions, making them highly susceptible to infections with nosocomial infections. This study investigated the prevalence of *bla*TEM, *bla*SHV, and *bla*PER genes and examined antimicrobial resistance profiles in *E. coli* isolates obtained from renal failure patients in Shahid Rajaee hospital, Gachsaran, Iran.

Materials & Methods: A cross-sectional analysis was conducted on 311 urine specimens collected from patients with renal failure. Among these specimens, 100 *E. coli* isolates were confirmed through biochemical identification. Antimicrobial susceptibility testing was carried out using the disk diffusion method, ESBLs production was confirmed by combined disk assay, and the presence of resistance genes was assessed using PCR.

Results: ESBLs activity was detected in 39% of isolates. Among ESBLs-producing strains, *bla*TEM was identified in 75%, *bla*SHV in 58.3%, and *bla*PER in 25%. The highest bacterial resistance was against nalidixic acid (61%), while the greatest susceptibility was to imipenem (92%). There is a statistically significant relationship between antibiotic resistance to the cefotaxime and different age groups of people. Also, 14% of isolates show multidrug resistance (MDR).

Conclusion: The high frequency of resistance determinants in *E. coli* isolated from patients with renal failure emphasizes the importance of rational antibiotic prescribing and strict infection control measures to reduce the spread of resistant strains in clinical settings.

Keywords: Renal Failure, ESBL producing *E. coli*, *bla*SHV, *bla*TEM, *bla*PER.

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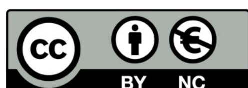

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Introduction

Escherichia coli is an opportunistic, Gram negative bacterium that is typically found in the human gastrointestinal microbiota. However, certain conditions, such as compromised host immunity, frequent hospitalizations, invasive procedures like catheterization, or improper antibiotic therapy, can cause *E. coli* to transition from a harmless commensal microorganism to a pathogenic one. This shift can result in a range of infections, including urinary tract infections, sepsis, neonatal bacterial meningitis, respiratory tract infections, and nosocomial infections (1,2). Successful infection in humans requires *E. coli* to express multiple genetic and phenotypic virulence factors. Key examples include fimbriae and pili that mediate adherence to host cells, capsules that protect against phagocytosis, lipopolysaccharides that act as endotoxins and initiate inflammatory responses, flagella that enhance motility, and biofilms that support persistence in hostile environments (2,3). Genes such as *aggR*, *aatA*, *aap*, and *chuA*, which are typically located on plasmids or pathogenicity islands, play critical roles in encoding factors, that enable immune evasion and efficient colonization (4-6). Endotoxins also activate host signaling pathways, notably NF-κB, driving inflammatory cascades can lead to tissue damage and septic shock. Additionally, biofilm formation is a major virulence trait that enhances resistance to antibiotics and host immune defenses, especially in catheterized patients (7). In recent years, the global increase in antibiotic resistance among Gram-negative bacteria, particularly *E. coli*, has become a significant public health concern (6,8). Resistance mechanisms include reduced outer membrane permeability, activation of efflux pumps, modifications of antibiotic target sites, and most importantly, the production of beta-lactamase enzymes. Among these, extended-spectrum beta-lactamases (ESBLs) are particularly critical. ESBLs hydrolyze and deactivate a wide range of beta-lactam antibiotics, such as penicillin, first to third-generation cephalosporins (e.g., cefotaxime and ceftriaxone), and aztreonam, thus severely limiting treatment options (9,10). The genes encoding these enzymes, particularly *bla*TEM, *bla*SHV, and *bla*PER, are commonly located on conjugative plasmids. These plasmids facilitate rapid dissemination across bacterial populations via horizontal gene transfer mechanisms such as conjugation, transformation, and transduction (9). The *bla*TEM gene, one of the earliest identified ESBLs genes, encodes TEM-type beta-lactamases that have expanded substrate profiles through point mutations. This confers resistance to newer cephalosporins (11). Similarly, *bla*SHV, initially linked with narrow-spectrum beta-lactamases, has evolved into SHV-type ESBLs with enhanced activity against third-generation cephalosporins (12,13). Although less frequent, the *bla*PER gene is increasingly reported; it encodes PER-type ESBLs highly effective against cefotaxime and is frequently associated with multidrug resistant strains in nosocomial settings (14). These plasmid-borne genes are frequently found alongside other resistance determinants, such as those for aminoglycosides and fluoroquinolones, further exacerbating the clinical challenge of multidrug resistance (8). The swift spread of ESBLs genes in hospitals, fueled by the misuse and/or overuse of antibiotics and patient-to-patient transmission, has significantly raised the prevalence of resistant *E. coli* strains. This, in turn, complicates treatment management and contributes to

treatment failures (9,10). Moreover, plasmid mobility allows the transfer of these genes not only among *E. coli* strains but also to other Gram-negative pathogens, amplifying the global challenge of antimicrobial resistance (15). Renal failure patients are particularly susceptible to infections caused by resistant organisms due to repeated urinary catheterization, dialysis, kidney transplantation, and prolonged hospitalizations. Reports show significantly higher resistance rates to antibiotics such as ampicillin, nalidixic acid, and certain fluoroquinolones in this population compared to the general population (16,17). The selective pressure exerted by excessive antibiotic use in hospital environments contributes to the emergence of resistant strains, reducing treatment efficacy and increasing mortality from resistant infections (16,18). Therefore, detecting resistant strains through phenotypic approaches (e.g., disk diffusion assays) and confirming resistance genes using molecular techniques such as PCR is essential to effectively managing nosocomial infections. Findings from these investigations provide critical guidance for antibiotic selection, help prevent the spread of resistance determinants, and strengthen infection control policies (19). Due to the rising prevalence of antibiotic resistance in *E. coli*, especially in high-risk renal failure patients, it is crucial to accurately determine resistance profiles and detect ESBLs -encoding genes (*bla*TEM, *bla*SHV, *bla*PER) in clinical isolates. This study aimed to examine antibiotic resistance patterns and evaluate the prevalence of these genes in *E. coli* strains isolated from renal failure patients.

Materials and Methods

A) Study Population: This descriptive cross sectional laboratory study was conducted in 2018 at the Microbiology Department of the Shahid Rajaee hospital in Gachsaran, Iran. The study population consisted of patients with renal failure who sought medical care for clinical symptoms of urinary tract infections (UTIs) and had submitted urine samples for microbiological analysis. Inclusion criteria included a confirmed diagnosis of renal failure by a specialist (including dialysis patients, kidney transplant recipients, or those with chronic renal insufficiency) and the presence of clinical UTI symptoms such as dysuria, frequent urination, cloudy or foul-smelling urine, fever, or flank pain. Patients who had taken antibiotics within the previous 72 hours or had underlying non-renal conditions (e.g., active malignancies) were excluded.

B) Sample Collection: Samples were collected using a non-probability convenience sampling method. A total of 311 urine samples were obtained under sterile conditions in accordance with standard protocols. All samples were immediately transported to the microbiology laboratory for bacterial culture and analysis (20).

C) Isolation and Identification of E. coli Isolates: Urine samples were cultured on selective media, including MacConkey agar and blood agar, and incubated at 37°C for 18–24 hours. Suspected colonies were evaluated based on color, shape, and morphology. Final identification of *E. coli* isolates was achieved through standard biochemical tests, including indole, citrate, urease, and IMViC assays. Confirmed *E. coli* isolates were stored in BHI broth with 15% glycerol at -80°C for further analysis (21).

D) Antibiotic Susceptibility Testing: Antibiotic resistance patterns of *E. coli* isolates were determined using the standard disk diffusion method on Mueller-Hinton agar, following the

guidelines by the CLSI criteria (22). Bacterial colonies were suspended in sterile saline, adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL), and then uniformly spread on Mueller-Hinton agar. Antibiotic disks, including imipenem (10 µg), nalidixic acid (30 µg), aztreonam (30 µg), cefotaxime (30 µg), and ceftazidime (30 µg), were placed on the agar surface. Plates were incubated at 37°C for 18–24 hours, and the diameters of inhibition zones were measured in millimeters. Based on CLSI interpretive tables, results were interpreted as susceptible (S), resistant (R), or intermediate (I). The standard strain *E. coli* ATCC 25922 was employed for quality control. Multidrug resistance (MDR) was defined as resistance to three or more antibiotic classes (22, 23).

E) Phenotypic Detection of ESBLs-Producing Isolates: Phenotypic detection of ESBLs production in *E. coli* isolates was performed using the combined disk test, following CLSI 2018 protocols. Isolates showing resistance to third-generation cephalosporins (e.g., cefotaxime or ceftazidime) were chosen for screening. Bacterial suspensions (adjusted to 0.5McFarland standard) were spread on Mueller-Hinton agar. Disks containing ceftazidime (30 µg) or cefotaxime (30 µg) alone, as well as in combination with clavulanic acid (10/30 µg), were placed 25–30 mm apart. After incubation at 37°C for 18–24 hours, an increase in the inhibition zone diameter of ≥5 mm around the combination disk compared to the cephalosporin disks alone, confirmed ESBLs production. *Klebsiella pneumoniae* ATCC 700603 (ESBLs positive) and *E. coli* ATCC 25922 (ESBLs negative) were used as positive and negative controls, respectively (23).

F) Frequency pattern of resistance genes:

1) DNA Extraction: Genomic DNA was extracted from *E. coli* isolates using a rapid boiling method. Fresh colonies were suspended in 500 µL of sterile TE buffer or deionized water, vortexed, and heated at 95–100°C for 10 minutes to lyse cells and release DNA. Samples were centrifuged at 12,000 rpm for 10 minutes, and the supernatant containing genomic DNA was transferred to new microtubes and stored at 4°C until PCR analysis. DNA quality and quantity were randomly assessed using 1% agarose gel electrophoresis and a NanoDrop spectrophotometer (with a 260/280 nm absorbance ratio of 1.8–2.0) (24).

2) Polymerase Chain Reaction (PCR): The presence of *bla*TEM, *bla*SHV, and *bla*PER genes was investigated using PCR with specific primers (Table 1). The PCR reaction mixture included buffer, $MgCl_2$, dNTPs, specific primers, Taq polymerase, and template DNA. PCR products were analyzed through 1.5% agarose gel electrophoresis, with expected band sizes indicating the presence of the genes. Positive controls for the study included *Klebsiella pneumoniae* ATCC 700603 (*bla*SHV), *E. coli* ATCC 35218 (*bla*TEM), and *Klebsiella pneumoniae* CS1711 (*bla*PER). A

Table 1. PCR Primer Sequences and Cycling Conditions.

Gene		Primmer Sequence 5' ⟶ 3'	Ref	Annealing Temperature (^{0}C)	PCR Condition	Reaction Mixture
*bla*PER	Forward	AATTTGGGCTTAGGGCAGAA	(25)	58	Initial denaturation: 95°C (10 min); 35 cycles of denaturation (95°C/40 s), annealing temperature (45 s), extension (72°C/60 s); Final extension (72°C/10 min)	2.5 µL PCR buffer (2x), 0.75 µL MgCl2, 0.5 µL dNTP, 0.25 µL Taq DNApolymeras, 2 µL DNA and up to 25 µL dH2O
	Reverse	ATGAATGTCATTATAAAAGC				
*bla*SHV	Forward	GGTTATGCGTTATATTCGCC	(25)	55		
	Reverse	TTAGCGTTGCCAGTGCTC				
*bla*TEM	Forward	ATGAGTATTCAACATTTCCG	(25)	60		
	Reverse	CTGACAGTTACCAATGCTTA				

master mix without template DNA was utilized as the negative control.

G) Statistical Analysis: Phenotypic and genotypic data were analyzed using SPSS version 20. Associations between qualitative variables were evaluated using chi-square and Fisher's exact tests, with statistical significance set at $p < 0.05$.

Results

A) Demographic Characteristics of the Study Population: A total of 311 urine samples were collected from patients with renal failure. Among the samples, 100 clinical *E. coli* isolates were identified through phenotypic characteristics and biochemical tests. Out of these isolates, 74 (74%) were obtained from female patients, and 26 (26%) were from male patients. The highest prevalence of *E. coli* was observed in the 25–32 age group, while the lowest prevalence was noted in the 49–56 age group. Detailed demographic data are presented in Table 2.

Table 2. Demographic Characteristics of the Study Population.

Characteristics		N (%)
Sex	Female	74 (74%)
	Male	26 (26%)
Age	8-16	4 (4%)
	17-24	14 (14%)
	25-32	32 (32%)
	33-40	19 (19%)
	41-48	13 (13%)
	49-56	1 (1%)
	57-64	17 (17%)
Marital Status	Married	71 (71%)
	Single	29 (29%)
Previous Medical History	+	21 (21%)
	-	79 (79%)

B) Antibiotic Susceptibility Testing by Disk Diffusion: The antibiotic susceptibility profiles of the *E. coli* isolates were evaluated using the disk diffusion method, and the results are summarized in Figure 1. The highest level of resistance was seen against nalidixic acid (61%), while the highest susceptibility was found for imipenem (92%). Furthermore, 14 isolates (14%) were classified as multidrug-resistant (MDR), which is defined as resistance to three or more classes of antibiotics.

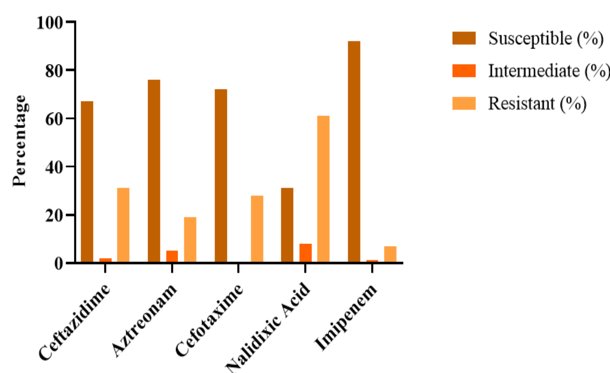

Fig 1. Antibiotic Susceptibility Profiles of *E. coli* Isolates.

C) Phenotypic Detection of ESBLs: Phenotypic detection of ESBLs production was carried out using the combined disk test (CDT), which identified 39 isolates (39%) as ESBLs producers. Out of these, 12 isolates (30.7%) were confirmed to be ESBLs-positive, showing an increase in the inhibition zone diameter of ≥5 mm for disks containing clavulanic acid compared to cephalosporin disks alone, while the remaining 27 isolates (69.3%) did not exhibit ESBLs activity. Among the positive cases, 6 isolates (6%) produced ESBLs against one screening antibiotic, 9 isolates (9%) against two antibiotics, and 24 isolates (24%) against three antibiotics. In contrast, 61 isolates (61%) demonstrated no ESBLs production.

D) Genotypic Prevalence of Beta-Lactamase Genes: PCR analysis was conducted on the 12 ESBLs-positive isolates to detect the presence of *blaTEM*, *blaSHV*, and *blaPER* genes. The

blaTEM gene was identified in 9 isolates (75%), *blaSHV* in 7 isolates (58.3%), and *blaPER* in 3 isolates (25%) (Figure 2). These results indicate that *blaTEM* was the most prevalent resistance gene, while *blaPER* was the least common. The presence of these genes, often carried on transferable plasmids, highlights a significant risk for the rapid dissemination of antimicrobial resistance in hospital settings.

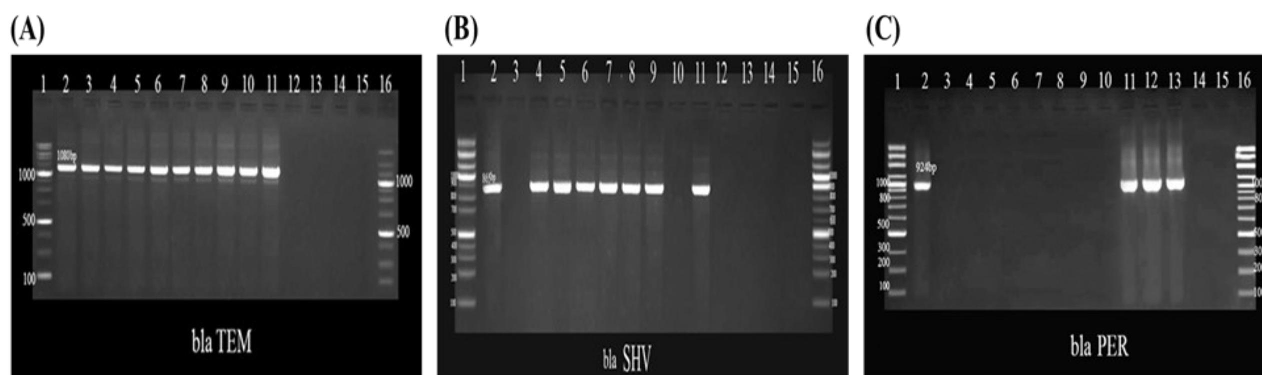

Fig 2. Agarose gel electrophoresis (1.5%) for detection of β-lactamase genes *bla*TEM, *bla*SHV, and *bla*PER in clinical *E. coli* isolates. Columns: 1,16, molecular ladder; 2, positive control; 15, negative control; 3-14, clinical samples. (A) *bla*TEM amplification in columns 3-11. (B) *bla*SHV amplification in columns 4-9 and 11. (C) *bla*PER amplification in lanes 11-13.

Discussion

The rising prevalence of ESBLs-producing bacterial strains, especially *E. coli*, presents a significant challenge in treating infections caused by Gram negative bacteria. These enzymes provide resistance to a broad spectrum of β-lactam antibiotics, including third generation cephalosporins like ceftazidime, cefotaxime, and cefepime (26,27). In this study, a high prevalence of resistance to commonly used antibiotics was observed among *E. coli* isolates, highlighting the clinical and public health implications of this issue. It was found that 39% of the isolates were ESBLs producers, a rate which considered moderate to high compared with the reports from other countries. A wide variation in the prevalence of resistance to commonly used antibiotics has been observed globally, with reported figures ranging from 2-10% in the United States and 13.3% in Lebanon to 36.8% in Nigeria, 62% in France, and 68% in India. These variations underscore the global distribution of ESBLs producing bacteria and regional differences in their prevalence. Factors contributing to these differences include variations in antibiotic prescribing practices, infection control policies, types of clinical specimens analyzed, characteristics of the study population, and the timing of the studies (26,28,29). In this study, antimicrobial resistance patterns showed that the highest bacterial resistance against nalidixic acid (61%), followed by ceftazidime (31%), cefotaxime (28%), and aztreonam (19%). In contrast, only 7% of isolates showed resistance to imipenem. Imipenem, as a carbapenem, is typically the preferred treatment for ESBLs producing bacteria, so the relatively low resistance rate is significant. However, even this level of resistance is worrying when compared to other studies that reported rates of zero or less than 5% (9,29,30). This finding may indicate an emerging trend of carbapenem resistance in the studied region, which could have serious implications given the limited therapeutic options available once carbapenem

resistance becomes widespread. Another key observation was that 14% of the isolates were classified as MDR, defined as resistance to three or more antibiotic classes. The presence of MDR strains significantly limits therapeutic options and complicates the clinical management of bacterial infections, particularly in vulnerable populations such as patients with chronic kidney disease. These patients are at heightened risk of acquiring resistant infections due to frequent hospitalizations, extensive use of invasive medical devices such as catheters, and repeated or prolonged antibiotic courses. Consequently, routine surveillance of antimicrobial resistance patterns in this patient group is critical for effective infection control and for guiding appropriate empirical therapy (31,32).

At the molecular level, the β-lactamase genes *bla*TEM, *bla*SHV, and *bla*PER were investigated, with frequencies of 75%, 58.3%, and 25%, respectively. These findings align with some previous studies, such as the one by Zambri *et al*., in Malaysia (33), but differ considerably from some others (34,35). These discrepancies may be attributed to variations in epidemiological factors, sample sources, molecular detection methods, and the extent of antibiotic use across different regions and healthcare systems (22).

The coexistence of ESBLs production and various resistance determinants poses additional diagnostic and therapeutic challenges. For example, ESBLs production can cause misleading results in antibiotic susceptibility testing, potentially leading to inappropriate therapy. Therefore, it is strongly recommended that clinical microbiology laboratories use confirmatory phenotypic assays in conjunction with molecular diagnostic techniques to ensure accurate detection of ESBLs producing organisms (36,37). Furthermore, the horizontal transfer of resistance genes via plasmids plays a critical role in the dissemination of ESBLs (38-40). Many of these plasmids carry multiple resistance determinants, which accelerates the spread of antibiotic resistance within and between bacterial species. Collectively, these findings underscore the urgent need for continuous surveillance of resistance mechanisms, the implementation of rational antibiotic stewardship programs, and stringent infection control measures. Additionally, the increasing prevalence of carbapenem resistance, although currently low, necessitates close monitoring to prevent its establishment and subsequent impact on treatment outcomes. Targeted molecular surveillance and efforts to curb the horizontal transmission of antibiotic resistance genes need to be essential components of public health strategies aimed at controlling the spread of ESBLs-producing *E. coli.*

Conclusion

The results of this study emphasize the significant contribution of ESBLs-producing *E. coli* to healthcare-associated infections, especially in patients with chronic kidney disease. Therefore, these results emphasize the importance of robust infection control, continuous antimicrobial resistance monitoring, and careful antibiotic stewardship. It is crucial to identify resistant strains and their genetic determinants to guide the selection of effective treatment regimens and prevent further resistance spread. Therefore, antibiotic administration should be precise, closely monitored, and carried out within well-structured stewardship programs that need to be prioritized and implemented with great care.

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Conflict of interest

The authors declare no competing interests.

Ethical considerations

This study was approved by the Research Ethics Committee of Islamic Azad University of Gachsaran (Ethical code 9706950161357). The study protocol adhered to the ethical guidelines of the 2008 Declaration of Helsinki.

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