

DETECTION OF *blaTEM* GENE IN *ESCHERICHIA COLI* ISOLATED FROM CLINICAL SAMPLES

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Abstract

Background: UTIs are one of the most common bacterial infections seen in clinical settings and antimicrobial resistance has become more of a therapeutic challenge. One of the most important cause of UTI is uropathogenic *Escherichia coli* and β -lactam production called extended-spectrum β -lactamase (ESBL) may significantly decrease the effectiveness of β -lactam antibiotics. *blaTEM* is a relevant determinant that is related to resistance to β -lactam antibiotics and has been found in clinical Enterobacteriaceae strains. As far as Pakistan is concerned the distribution of this gene on the UTI associated isolates is still limited.

Objective: The objective of this study was to determine the prevalence of *blaTEM*-mediated antimicrobial resistance and characterize the antimicrobial susceptibility pattern of *Escherichia coli* isolates recovered from urine samples of patients with urinary tract infections (UTIs) in tertiary-care hospitals in Lahore, Pakistan.

Methods: A total of 52 *Escherichia coli* isolates were recovered from urine samples of UTI patients attending tertiary-care hospitals in Lahore, Pakistan. The isolates were cultured and identified using MacConkey and Eosin Methylene Blue (EMB) agar. Antimicrobial susceptibility testing was performed against 24 antibiotics using the Kirby-Bauer disk diffusion method. Genomic DNA was extracted by heat lysis, and conventional PCR was employed to detect the *blaTEM* β -lactamase resistance gene. PCR products were visualized by agarose gel electrophoresis. The association between *blaTEM* gene detection and patient gender was assessed using the chi-square test.

Results: The *blaTEM* gene was detected in 14 of 52 (26.9%) *E. coli* isolates, while 38 (73.1%) were negative. The prevalence of *blaTEM*-positive isolates was higher among females (11/29; 37.9%) than males (3/23; 13.0%), with a statistically significant association between gender and *blaTEM* detection ($p = 0.04$). Most isolates were obtained from patients aged ≤ 20 years (24/52; 46%), followed by those aged 21–40 years (12/52; 23%) and 61–80 years (10/52; 19%), while 4 (7.6%) were aged 41–60 years; age data were unavailable for two patients. High resistance rates were observed

against cefuroxime (90%), ampicillin (88%), nitrofurantoin (87%), and cefotaxime (82%).

Conclusion: The study identified *bla*TEM-mediated resistance in uropathogenic *Escherichia coli*, with high resistance to broad-spectrum cephalosporins and ampicillin, particularly among female patients. These findings highlight the need for continuous molecular surveillance and judicious antibiotic use to support effective treatment and minimize antimicrobial resistance and therapeutic failure.

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections affecting individuals of all age groups and range from uncomplicated lower urinary tract infections to complicated infections requiring hospitalization. The increasing prevalence of antimicrobial resistance (AMR) among uropathogens has made the treatment of UTIs increasingly challenging. *Escherichia coli* (*E. coli*) is the predominant uropathogen associated with both community-acquired and healthcare-associated UTIs. Although commonly considered part of the normal intestinal microbiota, pathogenic strains can acquire virulence and antimicrobial resistance determinants that facilitate urinary tract colonization and persistence (Zhou et al., 2023; Jafri et al., 2014; Ramos et al., 2020).

The emergence of AMR is driven by bacterial evolution, antimicrobial selection pressure, horizontal gene transfer, mobile genetic elements, and inappropriate or excessive antibiotic use (Partridge et al., 2018; Hutchings et al., 2019; Larsson & Flach, 2022; Tang et al., 2023). Bacteria can develop resistance through several mechanisms, including modification of drug targets, reduced membrane permeability, active efflux, and enzymatic degradation of antimicrobial agents (Kapoor et al., 2017).

β -lactam antibiotics remain important for treating bacterial infections; however, their effectiveness is increasingly compromised by β -lactamase enzymes. Extended-spectrum β -lactamases (ESBLs) hydrolyze a broad range of β -lactam antibiotics, limiting therapeutic options. The *bla*TEM gene family is one of the well-characterized β -lactamase resistance determinants in Enterobacteriaceae. TEM-type enzymes have undergone extensive

evolutionary diversification, resulting in variations in their substrate spectrum and clinical resistance profiles (Yazdi et al., 2011; Salverda et al., 2010; Matlock et al., 2025). The location of these resistance genes on plasmids and other mobile genetic elements can facilitate their horizontal transmission among bacterial populations (Partridge et al., 2018; Heffron et al., 1975).

The prevalence of *bla*TEM among uropathogenic *E. coli* varies considerably across geographical regions. Studies from Iran and India have reported *bla*TEM prevalence of approximately 51% and 49.4%, respectively, among relevant urinary or ESBL-producing isolates (Barzegar et al., 2022; Verma et al., 2023), while some Indian studies have reported prevalence exceeding 50% (Biswas et al., 2025). Such variation may reflect differences in patient populations, antimicrobial exposure, healthcare settings, laboratory methods, and geographical factors.

In Pakistan, considerable antimicrobial resistance has been reported among urinary *E. coli* isolates, highlighting the need for local molecular surveillance of resistance determinants (Niaz et al., 2024; Ahmed et al., 2022; Jafri et al., 2014). However, data regarding the occurrence of *bla*TEM among uropathogenic *E. coli* isolates from tertiary-care hospitals in Lahore remain limited. Therefore, the present study aimed to determine the prevalence of the *bla*TEM gene and characterize the antimicrobial susceptibility pattern of *E. coli* isolates recovered from UTI patients in tertiary-care hospitals in Lahore, Pakistan.

Objective:

The study aimed to determine the isolation rate and antimicrobial resistance pattern of *E. coli* from urine samples of UTI patients and assess the association of *bla*TEM detection with patients' age and gender.

Methodology

Study design and setting

This laboratory-based cross-sectional study was conducted in three tertiary-care hospitals in Lahore, Pakistan. Urine specimens were collected from patients diagnosed with UTI over an approximately four-month study period and transported to the microbiology laboratory under appropriate biosafety and ethical guidelines. A total of 52 clinical *E. coli* isolates were included in the study.

Study population and eligibility

Patients of all age groups with clinically suspected or diagnosed UTI were included. Urine samples yielding *E. coli* were eligible for further analysis. Samples from patients without UTI, isolates other than *E. coli*, and patients aged >80 years were excluded. Two records with missing age information were excluded from demographic analysis.

Isolation and identification of *E. coli*

Urine specimens were aseptically streaked onto MacConkey agar and incubated at 37°C. Suspected lactose-fermenting colonies were subsequently subcultured on Eosin Methylene Blue (EMB) agar. Colonies showing a characteristic dark center with a green metallic sheen were considered presumptive *E. coli* (Lal A & N, 2007). Isolated *E. coli* colonies were subjected to antimicrobial susceptibility testing and molecular analysis.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar according to CLSI guidelines (Clinical, 2011). Antibiotic disks were applied to a standardized bacterial lawn and

incubated at 37°C. The zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant according to CLSI criteria. A panel of 24 antimicrobial agents, including ertapenem, cefixime, nitrofurantoin, amoxicillin-clavulanic acid, ceftazidime, fosfomycin, ciprofloxacin, polymyxin B, cefotetan, meropenem, gentamicin, amikacin, imipenem, cefepime, ampicillin, amoxicillin, cefuroxime, cefotaxime, piperacillin-tazobactam, ornidazole, and ofloxacin, was tested against the *E. coli* isolates.

Genomic DNA Extraction

Genomic DNA was extracted from individual *E. coli* colonies using the heat-lysis method. Briefly, a bacterial colony was suspended in sterile injection water and heated at approximately 95°C for 10 minutes, followed by centrifugation at 14,000 rpm for 5–10 minutes. The resulting supernatant containing genomic DNA was collected and used as the template for PCR amplification. The heat-lysis method was selected because of its simplicity, rapidity, and cost-effectiveness, particularly for resource-limited laboratories (Dashti et al., 2009).

PCR amplification of *bla*TEM

The *bla*TEM target has been detected by conventional PCR. The reaction volume was 25 µL and contained 22 µL of PCR Master mix, 0.5 µL forward primer, 0.5 µL reverse primer and 2 µL of DNA template as per the thesis (Chaudhary et al., 2023). Thermal cycling were done as follows: denaturation (95°C, 5 minutes) followed by 35 cycles of denaturation (95°C, 45 seconds), annealing (57°C, 1 minute), and extension (72°C, 1 minute); extension (72°C, 5 minutes) followed by hold (4°C). In the study, the size of the amplicon mentioned is 516 bp.

Agarose-gel electrophoresis

PCR products were run on an agarose-gel. The 1% agarose gel and 0.5× TBE buffer is described, along with taking the PCR product and adding in loading dye, plus adding in a DNA ladder for size comparison. The proteins were separated by electrophoresis at 120 V for about 90 minutes

then illuminated with ultraviolet radiation. Samples giving a product of 516-bp were considered as *blaTEM* positive.

Statistical analysis

Data was tabulated and organized in Microsoft-Excel software based on the demographic and laboratory observations done. The age groups, gender distribution, *blaTEM* status and antimicrobial susceptibility were summarized by descriptive analysis. To assess gender differences in blade yielding (*blaTEM* detection), the association was tested using a chi-square test. A p-value < 0.05 was considered statistically significant. The current study reported a p value of 0.04 for the relationship of gender and detection of *blaTEM*.

Demographic profile

Fifty-two *E. coli* isolates were obtained from UTI patients for final assembling of the data. Twenty-three of the isolates (44.2%) came from male patients while 29 isolates (55.8%) came from female patients. The age distribution showed that 24 isolates (46%) belonged to the 3-month-to-20-year group, 12 (23%) to 21–40 years, 4 (7.6%) to 41–60 years, and 10 (19%) to 61–80 years. There was no data on age in two records. The biggest of the age groups represented were then the youngest.

Prevalence of *blaTEM*

The 14 isolates harbouring *blaTEM* target were identified by PCR and gel electrophoresis, with a prevalence of 26.9%. Seven of the 38 (73.1%) were negative. The amplified product was said to be 516

bp. Therefore, while most of the isolates were negative to the target, more than a quarter of the isolates were positive for specific resistance determinant in this investigation.

Association between *blaTEM* detection and gender

The prevalence of *blaTEM*-positive isolates was higher among females than males. Among the 29 female isolates, 11 (37.9%) were *blaTEM*-positive, compared with 3 (13.0%) of 23 male isolates. A chi-square test showed a statistically significant association between gender and *blaTEM* detection ($p = 0.04$), as the p-value was below the significance level of 0.05. However, this association does not imply a causal relationship between gender and *blaTEM* carriage.

Antimicrobial susceptibility profile

The antimicrobial susceptibility profile showed considerable variation among the tested agents. The highest resistance rates were observed for cefuroxime (90%), ampicillin (88%), nitrofurantoin (87%), cefotaxime (82%), amoxicillin (76%), colistin (73%), cefixime (62%), ceftazidime (60%), and cefepime (53%). In contrast, resistance was comparatively low to ertapenem (2%), cefotetan (2%), imipenem (7%), meropenem (8%), and levofloxacin (9%). The isolates also showed relatively high susceptibility to cefotetan (81%), sulbactam (75%), meropenem (75%), polymyxin B (75%), and imipenem (73%). These findings indicate substantial resistance to several commonly used antibiotics among the isolated uropathogenic *E. coli*.

Table 1. Age and gender distribution reported in the study.

Age group	Male	Female	Total	Percentage
3 months–20 years	11	13	24	46%
21–40 years	3	9	12	23%
41–60 years	3	1	4	7.6%
61–80 years	5	5	10	19%

Table 2. Distribution of *blaTEM* detection by gender.

Gender	<i>blaTEM</i> positive	<i>blaTEM</i> negative	Total
Male	3 (13.0%)	20 (87.0%)	23 (44.2%)

Female	11 (37.9%)	18 (62.1%)	29 (55.8%)
Overall	14 (26.9%)	38 (73.1%)	52 (100%)

Table 3. Antimicrobial susceptibility percentages reported in the study.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Colistin	0	0	73
Nitrofurantoin	36	8	87
Sulbactam	75	2	10
Ertapenem	50	0	2
Cefixime	15	0	62
Clavulanate	17	2	56
Ceftazidime	15	0	60
Fosfomycin	35	0	31
Ciprofloxacin	11	6	40
Polymyxin B	75	0	58
Cefotetan	81	0	2
Meropenem	75	2	8
Gentamicin	30	0	23
Amikacin	30	5	23
Imipenem	73	1	7
Cefepime	9	5	53
Ampicillin	3	0	88
Amoxicillin	5	0	76
Cefuroxime	0	0	90
Cefotaxime	7	70	82
Tazobactam	25	7	55
Ofloxacin–Ornidazole	5	0	19
Ceftriaxone	3	1	40
Levofloxacin	0	0	9

Discussion

The present study investigated antimicrobial resistance and *bla*TEM detection among 52 uropathogenic *Escherichia coli* isolates from UTI patients in Lahore. The *bla*TEM gene was detected in 14 (26.9%) isolates, which was lower than rates reported in some regional studies from Iran and India (Barzegar et al., 2022; Verma et al., 2023). Such differences may reflect variations in study population, hospital setting, inclusion criteria, and antimicrobial exposure.

The prevalence of *bla*TEM was significantly higher among female isolates (37.9%) than male isolates (13.0%) ($p = 0.04$). However, this represents a sample-level association and does not establish causality. Most isolates were obtained from patients aged ≤ 20 years (46%), although this

distribution may reflect the sampled population rather than the general UTI burden.

High resistance was observed against cefuroxime (90%), ampicillin (88%), nitrofurantoin (87%), and cefotaxime (82%). Although ampicillin resistance is compatible with TEM-mediated β -lactam resistance, resistance cannot be attributed solely to *bla*TEM because other genetic and non-genetic mechanisms may contribute. Relatively high susceptibility to cefotetan, sulbactam, meropenem, polymyxin B, and imipenem highlights the importance of local susceptibility testing and antimicrobial stewardship.

PCR detection of the 516-bp *bla*TEM product confirms the presence of this resistance determinant but does not alone establish an ESBL phenotype. The study was limited by its small

sample size, restriction to Lahore, and investigation of only *blaTEM*. Larger multicenter studies incorporating additional resistance genes and sequencing are recommended to better characterize antimicrobial resistance and transmission in Pakistan.

Conclusion

The study detected the *blaTEM* gene in 14 (26.9%) of 52 uropathogenic *Escherichia coli* isolates from UTI patients in Lahore. *blaTEM* positivity was significantly higher among female isolates than male isolates ($p = 0.04$). High resistance was observed against commonly used antibiotics, particularly cefuroxime, ampicillin, nitrofurantoin, cefotaxime, amoxicillin, and colistin. These findings highlight the need for continuous antimicrobial resistance surveillance and judicious antibiotic use in UTI management.

Recommendations

A further study must be Conducted considering periodic molecular surveillance of *blaTEM* and other important ESBL resistance genes.

A future multicenter research can be Conducted across Pakistan for more representative findings.

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