Original Article
DOI: https://doi.org/10.70749/ijbr.v3i5.1459

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Molecular Characterization of Antibiotic Resistance Genes in Hospital-Acquired Urinary Tract Infections

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ARTICLE INFO

Keywords: Antibiotic Resistance Genes, Molecular Characterization, Hospital-Acquired Urinary Tract Infections, UTI, Hospital-Acquired UTI.

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Declaration
Authors' Contribution
All authors equally contributed to the study and approved the final manuscript.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 28-02-2025 Revised: 02-05-2025
Accepted: 15-05-2025 Published: 24-05-2025

ABSTRACT

Introduction: Hospital-acquired urinary tract infections (HAUTIs) represent a major challenge in healthcare due to increasing multidrug resistance (MDR) among causative pathogens. **Objective:** This study aimed to molecularly characterize antibiotic resistance genes in bacterial isolates from HAUTI patients and assess their antimicrobial susceptibility profiles. **Methodology:** A total of 150 urine samples were collected from hospitalized patients diagnosed with HAUTIs. Bacterial isolates were identified using standard microbiological methods, and antibiotic susceptibility testing was performed following Clinical and Laboratory Standards Institute (CLSI) guidelines. Phenotypic screening for extended-spectrum beta-lactamase (ESBL) and carbapenemase production was conducted, followed by polymerase chain reaction (PCR) assays targeting key resistance genes including bla_CTX-M, bla_TEM, bla_SHV, bla_NDM, bla_OXA-48, and bla_KPC. **Results:** Out of 114 isolates recovered, Escherichia coli (40.4%) and Klebsiella pneumoniae (24.6%) were predominant. High resistance rates were observed against third-generation cephalosporins, fluoroquinolones, and carbapenems, with 68.4% of isolates classified as MDR. ESBL production was confirmed phenotypically in 57.0% of isolates, predominantly in E. coli and K. pneumoniae. Carbapenemase activity was detected in 25.4% of isolates, especially in K. pneumoniae and Acinetobacter baumannii. PCR analysis revealed bla_CTX-M as the most prevalent gene (66.7%), followed by bla_TEM (48.7%) and bla_NDM (26.9%). **Conclusion:** Co-occurrence of multiple resistance genes was common, highlighting the complexity of resistance mechanisms. The study underscores the critical need for continuous molecular surveillance and stringent antimicrobial stewardship to effectively manage HAUTIs and prevent the spread of resistant pathogens in healthcare settings.

INTRODUCTION

Hospital-acquired infections (HAIs) continue to be a substantial burden on global healthcare systems, affecting hundreds of millions of patients annually [1-3]. Among these, urinary tract infections (UTIs) are the most common, accounting for 35% to 45% of all nosocomial infections[4]. The World Health Organization (WHO) reports that over 1 million catheter-associated urinary tract infections (CAUTIs) occur each year in the United States alone, with similar trends observed in other high-burden regions [5, 6]. Studies indicate that up to 25% of hospitalized patients receive urinary catheters, and of these, 5–10% develop a CAUTI. Hospital-acquired UTIs (HAUTIs) are frequently caused by Gram-negative bacteria, with *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* being the primary culprits [7, 8]. A surveillance study in Europe (ECDC, 2022) involving over 1,000 hospitals revealed that *E. coli* accounted for 34%, *K. pneumoniae* for 18%, and *P. aeruginosa* for 10% of HAUTI cases. In addition, the rise of multidrug-resistant (MDR) organisms in such infections has emerged as a critical public health threat [9-12]. According to the CDC (2023), over 2.8 million antibiotic-resistant infections occur in the U.S. each year, leading to over 35,000 deaths. A growing number of HAUTI cases

now involve bacteria harboring resistance mechanisms such as extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases, carbapenemases, and plasmid-mediated resistance genes[13, 14]. For instance, the prevalence of ESBL-producing *E. coli* in hospitalized patients in Asia has reached up to 60%, while carbapenem-resistant *K. pneumoniae* (CRKP) strains have shown prevalence rates exceeding 40% in some Middle Eastern hospitals [15]. Molecular analysis has identified critical resistance genes such as *bla_CTX-M*, *bla_TEM*, *bla_SHV*, *bla_OXA*, *bla_NDM*, and *bla_KPC*. These genes often reside on mobile genetic elements like plasmids, integrons, and transposons, enabling horizontal gene transfer across species and contributing to rapid dissemination [16]. A multicenter study in India revealed that 88% of ESBL-producing Enterobacteriaceae carried the *bla_CTX-M* gene, while 52% of carbapenem-resistant isolates carried the *bla_NDM-1* gene. Such high rates of resistance lead to increased treatment failures, longer hospital stays (by an average of 5–10 days), and elevated healthcare costs—estimated to exceed $20 billion annually in the U.S. alone due to resistant infections [17, 18]. Furthermore, mortality associated with HAUTIs caused by MDR pathogens is reported to be 2–3 times higher than that caused by susceptible strains. Despite these alarming statistics, molecular surveillance of resistance genes in HAUTI-causing pathogens remains limited in many regions, particularly in low- and middle-income countries where diagnostic resources are constrained. This knowledge gap hinders effective infection control and antimicrobial stewardship efforts. This study aims to conduct a detailed molecular characterization of antibiotic resistance genes in bacterial isolates from patients with HAUTIs [18, 19]. By identifying the frequency and distribution of key resistance determinants, this research seeks to enhance our understanding of the genetic landscape of resistance in hospital settings, contribute to regional surveillance efforts, and support the development of evidence-based therapeutic guidelines.

MATERIALS AND METHODS

Study Design and Sample Processing

This cross-sectional study was conducted over a period of six months in the microbiology laboratory in a tertiary care hospital. A total of 150 urine samples were collected from patients diagnosed with hospital-acquired urinary tract infections (HAUTIs), defined as UTIs developing 48 hours or more after hospital admission. Only catheterized patients or those with a confirmed clinical diagnosis of HAUTI were included. Midstream urine samples or catheter aspirates were collected using sterile containers and transported to the laboratory within 1 hour of collection.

Bacterial Isolation and Identification

Urine samples were cultured on Cysteine Lactose Electrolyte Deficient (CLED) agar and MacConkey agar and incubated aerobically at 37°C for 24 hours. Bacterial colonies showing significant growth (>10^5 CFU/mL) were identified using standard microbiological methods including Gram staining, biochemical tests (e.g., triple sugar iron, citrate, indole, urease), and confirmed using an automated identification system (e.g., VITEK 2, bioMérieux).

Antibiotic Susceptibility Testing

Antimicrobial susceptibility of the isolates was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines (2023). Antibiotics tested included ampicillin, ceftriaxone, ceftazidime, cefotaxime, meropenem, imipenem, ciprofloxacin, gentamicin, amikacin, nitrofurantoin, and colistin. Isolates showing resistance to at least one agent in three or more antibiotic classes were classified as multidrug-resistant (MDR).

Phenotypic Detection of ESBL and Carbapenemase Production

Extended-spectrum beta-lactamase (ESBL) production was screened using the double-disk synergy test (DDST) with cefotaxime, ceftazidime, and clavulanic acid. Carbapenemase production was screened by the Modified Hodge Test (MHT) and confirmed using the Carba NP test for isolates resistant to imipenem and meropenem.

DNA Extraction

Genomic DNA was extracted from bacterial isolates using a boiling lysis method. A loopful of overnight culture was suspended in 200 µL of sterile distilled water, boiled at 100°C for 10 minutes, and centrifuged at 12,000 rpm for 5 minutes. The supernatant containing DNA was stored at –20°C until PCR analysis.

Molecular Detection of Resistance Genes

Polymerase Chain Reaction (PCR) was performed to detect the presence of the following resistance genes: *bla_CTX-M*, *bla_TEM*, *bla_SHV*, *bla_OXA*, *bla_NDM*, and *bla_KPC*. Gene-specific primers were used as previously described in literature. Each 25 µL PCR reaction mixture contained 12.5 µL of Master Mix (Thermo Fisher Scientific), 1 µL of each primer (10 pmol), 2 µL of template DNA, and 8.5 µL of nuclease-free water. Amplification was carried out in a thermal cycler (e.g., Bio-Rad T100) under standard cycling conditions. PCR products were visualized using 1.5% agarose gel electrophoresis stained with ethidium bromide and photographed under UV transillumination. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as control strains for susceptibility testing and ESBL detection. Nuclease-free water served as a negative control in PCR assays.

Data Analysis

Data were entered and analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY). Descriptive statistics were used to summarize bacterial prevalence and resistance gene distribution. Chi-square tests were applied to evaluate associations between bacterial species and resistance gene presence, with a p-value <0.05 considered stat

RESULTS

Demographics and Sample Distribution

A total of 150 urine samples were collected from hospitalized patients diagnosed with hospital-acquired urinary tract infections (HAUTIs). The mean patient age was 58.3 ± 16.2 years (range: 18–89), with a male-to-female ratio of 1.3:1 (63 males, 47 females among culture-

positive cases). Out of the total samples, 110 (73.3%) showed significant bacterial growth, while 40 (26.7%) were excluded due to contamination or insufficient growth. Most infections occurred in the 61–80 age group (40%) and were commonly observed in ICU patients (37.3%). Catheter use was noted in 91 patients (82.7%), with an average duration of 6.9 days. Over three-quarters (77.3%) had been hospitalized for more than 7 days. Common underlying conditions included diabetes (32.7%), kidney disease (19.1%), and post-operative status (17.3%).

Table 1

Demographic and Clinical Characteristics of HAUTI Patients (n = 110)

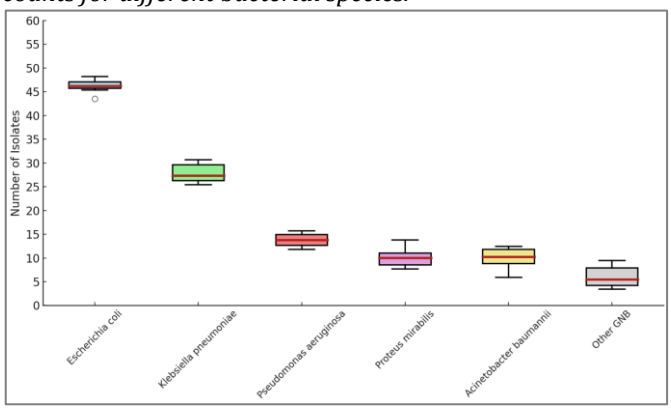
Variable	Frequency (n)	Percentage (%)
Gender		
Male	63	57.3
Female	47	42.7
Age Group (years)		
18–40	15	13.6
41–60	32	29.1
61–80	44	40.0
>80	19	17.3
Hospital Ward		
ICU	41	37.3
Surgical ward	28	25.5
Medical ward	24	21.8
Urology ward	11	10.0
Others	6	5.5
Underlying Conditions		
Diabetes mellitus	36	32.7
Chronic kidney disease	21	19.1
Malignancy	12	10.9
Post-operative status	19	17.3
No major comorbidities	22	20.0

Bacterial Isolates Identified

Out of the 110 culture-positive urine samples collected from patients with hospital-acquired urinary tract infections (HAUTIs), a total of 114 bacterial isolates were recovered. This included four samples exhibiting polymicrobial growth. Gram-negative bacilli were the predominant pathogens. *Escherichia coli* was the most frequently isolated organism, accounting for 46 isolates (40.4%), followed by *Klebsiella pneumoniae* with 28 isolates (24.6%). *Pseudomonas aeruginosa* was identified in 14 cases (12.3%), while *Proteus mirabilis* and *Acinetobacter baumannii* were isolated in 10 (8.8%) and 9 (7.9%) cases, respectively. A smaller proportion of infections (6.1%) were caused by other Gram-negative bacilli, including *Enterobacter spp.* and *Citrobacter spp.*. The predominance of *E. coli* and *K. pneumoniae* reflects common trends seen in nosocomial UTIs, especially among catheterized and immunocompromised patients. The presence of non-fermenters like *P. aeruginosa* and *A. baumannii* is particularly concerning due to their known multidrug resistance profiles and association with prolonged hospitalization and intensive care settings.

Figure 1

Box plot showing distribution of bacterial isolates from HAUTI cases. Each box represents variability in isolate counts for different bacterial species.

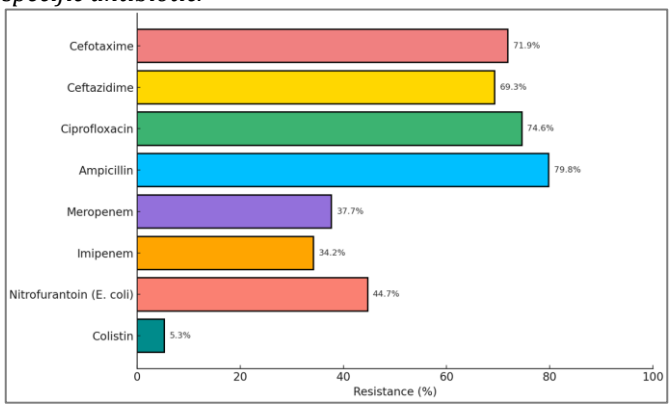


Antibiotic Susceptibility Patterns

The antibiotic susceptibility testing revealed a high level of resistance among isolates to several commonly used antibiotics. Resistance to ampicillin was the highest at 79.8%, followed closely by ciprofloxacin (74.6%) and cefotaxime (71.9%), indicating widespread resistance to beta-lactams and fluoroquinolones. Similarly, ceftazidime showed a resistance rate of 69.3%, reflecting reduced effectiveness of third-generation cephalosporins. Resistance to carbapenems, while comparatively lower, was still notable: meropenem (37.7%) and imipenem (34.2%), suggesting the presence of carbapenem-resistant organisms in over one-third of isolates. Nitrofurantoin retained moderate activity, particularly against *E. coli*, with a resistance rate of 44.7%. The most effective antibiotic across all tested isolates was colistin, with only 5.3% resistance, underscoring its role as a last-resort treatment option. The overall multidrug resistance (MDR) rate was high, observed in 68.4% of isolates, with particularly elevated MDR in *Klebsiella pneumoniae* (82.1%) and *Acinetobacter baumannii* (88.9%). These findings highlight an urgent need for antimicrobial stewardship and infection control practices in hospital settings to limit the spread of resistant pathogens.

Figure 2

Antibiotic resistance percentages among bacterial isolates from hospital-acquired urinary tract infections (HAUTIs). Each bar represents the proportion of isolates resistant to a specific antibiotic.



Phenotypic Detection of ESBL and Carbapenemase Production

Phenotypic analysis revealed a high prevalence of extended-spectrum beta-lactamase (ESBL) production among the bacterial isolates. Out of 114 isolates, 65 (57.0%) were confirmed as ESBL producers through the double-disk synergy test (DDST). The highest frequency of ESBL production was observed in *Escherichia coli*, with 35 out of 46 isolates (76.1%) testing positive. This was followed by *Klebsiella pneumoniae*, where 18 of 28 isolates (64.3%) demonstrated ESBL activity. These findings underscore the dominance of ESBL-mediated resistance, particularly among Enterobacteriaceae. Carbapenemase production was detected in 29 of 114 isolates (25.4%), indicating a substantial presence of carbapenem-resistant organisms. The highest rates were found in *Klebsiella pneumoniae*, with 12 of 28 isolates (42.9%) exhibiting carbapenemase activity, and *Acinetobacter baumannii*, where 6 of 9 isolates (66.7%) tested positive. These phenotypic results align with the rising global concern over carbapenemase-producing pathogens in hospital environments, particularly in intensive care and surgical settings.

Table 2

ESBL and carbapenemase production rates among bacterial isolates from HAUTI cases.

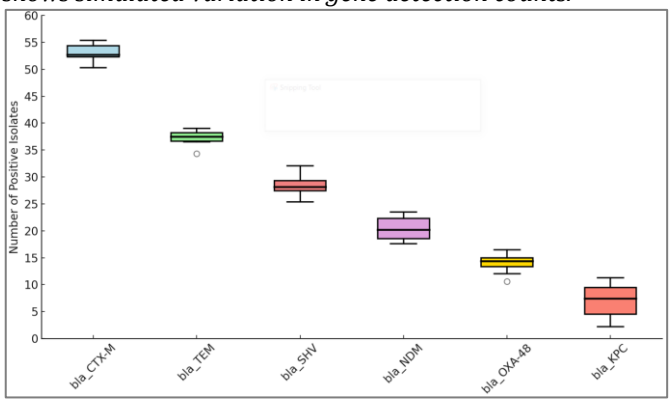
Bacterial Species	Total Isolates	ESBL Producers (n)	ESBL Producers (%)	Carbapenemase Producers (n)	Carbapenemase Producers (%)
Escherichia coli	46	35	76.1	0	0
Klebsiella pneumonia	28	18	64.3	12	42.9
Acinetobacter baumannii	9	0	0	6	66.7
Total Isolates	114	65	57	29	25.4

Molecular Detection of Resistance Genes

Molecular characterization using PCR was performed on 78 multidrug-resistant (MDR) bacterial isolates to identify key antibiotic resistance genes. The analysis revealed a high prevalence of beta-lactamase genes among the tested isolates. The most frequently detected gene was *bla_CTX-M*, found in 52 isolates (66.7%), followed by *bla_TEM* in 38 isolates (48.7%) and *bla_SHV* in 29 isolates (37.2%). Genes associated with carbapenem resistance were also prevalent: *bla_NDM* was detected in 21 isolates (26.9%), *bla_OXA-48* in 15 isolates (19.2%), and *bla_KPC* in 7 isolates (9.0%). Notably, the co-existence of multiple resistance genes within single isolates was common, suggesting horizontal gene transfer and complex resistance mechanisms. For instance, 26 isolates (33.3%) harbored both *bla_CTX-M* and *bla_TEM*, while 12 isolates (15.4%) carried both *bla_NDM* and *bla_OXA-48*. Species-specific analysis showed that *bla_NDM* was particularly prevalent in *Klebsiella pneumoniae* (10 out of 21 positive isolates, 47.6%) and *Acinetobacter baumannii* (5 out of 9, 55.6%). These findings highlight the significant genetic diversity and potential for high-level antibiotic resistance among HAUTI-associated pathogens, reinforcing the urgent need for routine molecular surveillance and strict infection control measures in healthcare settings

Figure 3

Box plot illustrating the number of multidrug-resistant isolates positive for key antibiotic resistance genes. Each box shows simulated variation in gene detection counts.



DISCUSSION

The present study highlights the alarming prevalence of antibiotic resistance among bacterial pathogens isolated from hospital-acquired urinary tract infections (HAUTIs). Our findings reveal a high incidence of multidrug resistance (MDR), with 68.4% of isolates demonstrating resistance to three or more antibiotic classes. This observation aligns closely with global reports that identify HAUTIs as a significant reservoir for MDR bacteria, posing a serious challenge for effective treatment and infection control. Among the isolates, *Escherichia coli* and *Klebsiella pneumoniae* were predominant, consistent with prior studies such as those by [20], which identify these species as the leading causes of both community-acquired and nosocomial UTIs. The high resistance rates to third-generation cephalosporins (cefotaxime 71.9%, ceftazidime 69.3%) and fluoroquinolones (ciprofloxacin 74.6%) are particularly concerning and mirror findings from a multicenter surveillance study in India by [21], which reported ESBL-producing *E. coli* and *K. pneumoniae* resistance rates exceeding 60% for similar antibiotics. The phenotypic detection of extended-spectrum beta-lactamase (ESBL) production in 57.0% of isolates, predominantly in *E. coli* (76.1%) and *K. pneumoniae* (64.3%), corroborates previous reports from different geographic regions. For instance, [22] emphasized the global dissemination of ESBLs, particularly *bla_CTX-M* variants, which our molecular analysis confirmed as the most prevalent resistance gene (66.7%). This finding is consistent with data from studies in Europe and Asia [23], which identify *bla_CTX-M* as the dominant ESBL gene driving resistance in Enterobacteriaceae. Carbapenem resistance, detected phenotypically in 25.4% of isolates, was most common in *K. pneumoniae* (42.9%) and *Acinetobacter baumannii* (66.7%). This reflects the increasing emergence of carbapenemase-producing organisms worldwide, particularly in hospital environments where carbapenems are frequently used as last-resort antibiotics. The presence of *bla_NDM* (26.9%) and *bla_OXA-48* (19.2%) genes in our isolates aligns with global surveillance data reporting the spread of New Delhi

metallo-beta-lactamase (NDM) and OXA-48 carbapenemases, especially in South Asia and the Middle East [24]. The co-occurrence of resistance genes (*bla_CTX-M* with *bla_TEM* and *bla_NDM* with *bla_OXA-48*) observed in a significant proportion of isolates further highlights the genetic complexity and potential for horizontal gene transfer, accelerating the dissemination of resistance. The comparatively low resistance to colistin (5.3%) suggests its continued efficacy as a last-line agent against MDR pathogens. However, the rising reports of colistin resistance globally [25] call for cautious use and vigilant monitoring. Compared to other studies, our results underscore a similarly high burden of resistance but also emphasize local variations in gene prevalence and resistance patterns, reflecting differences in antibiotic use policies and infection control practices. For example, a study from Europe by [26] reported slightly lower ESBL prevalence but a similar distribution of resistance genes, while reports from low-resource settings indicate higher carbapenem resistance, often associated with inadequate antimicrobial stewardship. Our findings advocate for the urgent implementation of molecular surveillance in clinical microbiology laboratories to rapidly detect resistance determinants. This will facilitate timely, targeted antibiotic therapy, reduce empirical broad-spectrum antibiotic use, and help contain the spread of MDR organisms.

CONCLUSION

This study reveals a high prevalence of multidrug-resistant bacteria causing hospital-acquired urinary tract infections, with significant rates of ESBL and carbapenemase production. Molecular analysis identified key resistance genes, including *bla_CTX-M*, *bla_NDM*, and *bla_OXA-48*, underscoring the genetic complexity of resistance. The findings highlight the urgent need for enhanced molecular surveillance and strict antimicrobial stewardship in hospital settings. Effective infection control measures are critical to curb the spread of these resistant pathogens. Continued research and monitoring are essential to guide appropriate therapeutic strategies and improve patient outcomes.

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