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# Phenotypic and Molecular Characterization of Carbapenem-Resistant Enterobacteriaceae Isolated from Urinary Tract Infections at a Tertiary Care Centre

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## ABSTRACT

**Background:** Carbapenem-resistant Enterobacteriaceae (CRE) represent a major therapeutic and public health challenge worldwide, particularly in urinary tract infections (UTIs), where limited antimicrobial options contribute to adverse clinical outcomes. Early detection of carbapenem resistance and its underlying mechanisms is critical for effective patient management and infection control.

**Objectives:** To determine the prevalence of carbapenem resistance among Enterobacteriaceae isolated from urine samples and to characterize carbapenem resistance using phenotypic methods and molecular detection of carbapenemase-encoding genes.

**Methods:** A cross-sectional study was conducted in a tertiary care teaching hospital from January 2022 to June 2025. Non-duplicate Enterobacteriaceae isolates obtained from urine samples were identified using standard microbiological techniques. Antimicrobial susceptibility testing was performed according to CLSI guidelines. Phenotypic detection of carbapenemase production was carried out using Carba NP and carbapenem inactivation methods. Molecular characterization of carbapenemase genes (bla_NDM, bla_VIM, bla_IMP, bla_KPC, and bla_OXA-48-like) was performed using multiplex polymerase chain reaction (PCR).

**Results:** Out of 350 Enterobacteriaceae isolates, carbapenem resistance was detected in a significant proportion. *Escherichia coli* was the predominant carbapenem-resistant isolate, followed by *Klebsiella pneumoniae*. Phenotypic carbapenemase activity was detected in the majority of carbapenem-resistant isolates. Molecular analysis revealed bla_NDM as the most prevalent carbapenemase gene, followed by bla_OXA-48-like. Co-production of carbapenemase genes was observed in a subset of isolates.

**Conclusion:** The study highlights a substantial burden of carbapenem-resistant uropathogenic Enterobacteriaceae in a tertiary care setting. Combined phenotypic and molecular diagnostic approaches are essential for early detection, appropriate antimicrobial therapy, and prevention of further dissemination of CRE.

**Keywords:** Carbapenem-resistant Enterobacteriaceae; Urinary tract infection; Carbapenemase; Phenotypic detection; bla_NDM; Molecular diagnosis

## INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections affecting individuals across all age groups and healthcare settings[1]. Members of the family *Enterobacteriaceae*, particularly *Escherichia coli* and *Klebsiella pneumoniae*, account for the majority of community-acquired and hospital-acquired UTIs[2].

The increasing prevalence of antimicrobial resistance among uropathogenic Enterobacteriaceae has emerged as a significant global concern[3]. Extended-spectrum β-lactamase (ESBL)–producing organisms have led to the widespread use of carbapenems as last-line therapeutic agents[4]. However, the emergence of carbapenem-resistant Enterobacteriaceae (CRE) has severely compromised the effectiveness of these drugs and limited available treatment options[5].

Carbapenem resistance in Enterobacteriaceae arises through multiple mechanisms, including reduced permeability due to porin loss, efflux pump overexpression, and enzymatic hydrolysis mediated by carbapenemases[6]. Carbapenemase

enzymes are classified into Ambler classes A, B, and D, with KPC, NDM, VIM, IMP, and OXA-48-like enzymes being the most clinically relevant and widely distributed[7].

Infections caused by CRE are associated with prolonged hospitalization, increased healthcare expenditure, and higher mortality rates compared to carbapenem-susceptible infections[5]. The World Health Organization has designated CRE as critical priority pathogens, emphasizing the urgent need for surveillance, early detection, and effective containment strategies[8].

India has reported a high prevalence of carbapenemase-producing Enterobacteriaceae, particularly those harboringbla_NDM and bla_OXA-48-like genes[9]. Despite this, data integrating phenotypic and molecular characterization of carbapenem resistance among uropathogens remain limited. The present study was undertaken to evaluate the prevalence of carbapenem resistance and to characterize the underlying resistance mechanisms among Enterobacteriaceae isolated from urinary tract infections in a tertiary care centre.

## MATERIALS AND METHODS

### Study Design and Setting
This cross-sectional laboratory-based study was conducted in the Department of Microbiology of a tertiary care teaching hospital over a period of January 2022 to June 2025.

### Study Population and Isolates
A total of 3617 urine samples received from outpatient and inpatient departments were processed during the study period. Non-duplicate Enterobacteriaceae isolates (n = 350) demonstrating significant bacteriuria (>$10^5$ CFU/mL) were included. Samples yielding mixed growth of more than three organisms were excluded.

### Identification of Isolates
Urine samples were cultured on 5% sheep blood agar and MacConkey agar using a semi-quantitative technique. Bacterial isolates were identified based on colony morphology, Gram staining, and standard biochemical tests[10].

### Antimicrobial Susceptibility Testing
Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines[11]. Carbapenem susceptibility was assessed using imipenem, meropenem, and ertapenem disks.

### Phenotypic Detection of Carbapenemase Production
Phenotypic detection of carbapenemase production was carried out using Carba NP and carbapenem inactivation methods, as recommended for routine laboratory screening[12].

### Molecular Detection of Carbapenemase Genes
All carbapenem-resistant isolates were subjected to multiplex polymerase chain reaction (PCR) for detection of bla_NDM, bla_VIM, bla_IMP, bla_KPC, and bla_OXA-48-like genes, following previously described protocols[13].

### Statistical Analysis
Data were entered into Microsoft Excel and analyzed using SPSS version 25.0. Results were expressed as frequencies and percentages.

## RESULTS

Among the 350 Enterobacteriaceae isolates, carbapenem resistance was detected in a notable proportion. *Escherichia coli* was the most frequently isolated carbapenem-resistant organism, followed by *Klebsiella pneumoniae*. Other Enterobacteriaceae, including *Citrobacter*, *Enterobacter*, and *Proteus* species, were isolated less frequently.

Phenotypic carbapenemase production was observed in the majority of carbapenem-resistant isolates. Molecular analysis demonstrated that bla_NDM was the most prevalent carbapenemase-encoding gene, followed by bla_OXA-48-like. A subset of isolates showed co-existence of multiple carbapenemase genes, suggesting plasmid-mediated dissemination of resistance determinants.

## DISCUSSION

The present study highlights the growing burden of carbapenem-resistant Enterobacteriaceae among uropathogens in a tertiary care setting. The predominance of *E. coli* and *K. pneumoniae* among CRE isolates is consistent with previous Indian and international studies[2],[14].

The high prevalence of bla_NDM observed in this study reflects its widespread dissemination in the Indian subcontinent[9]. The detection of bla_OXA-48-like genes further underscores the evolving complexity of carbapenem resistance mechanisms among Enterobacteriaceae[15].

The co-production of multiple carbapenemase genes within individual isolates is of particular concern, as it significantly limits therapeutic options and facilitates rapid horizontal transmission[7]. These findings emphasize the importance of combining phenotypic and molecular diagnostic methods for accurate detection of carbapenem resistance and effective infection control[8].

## CONCLUSION

This study demonstrates a significant prevalence of carbapenem-resistant Enterobacteriaceae among urinary tract infection isolates in a tertiary care hospital. The predominance of bla_NDM and bla_OXA-48-like genes highlights the urgent need for continuous surveillance, early laboratory detection, and robust antimicrobial stewardship programs to curb the spread of CRE.

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