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Molecular Epidemiology of Carbapenemase-Encoding Genes among Carbapenem-Resistant Enterobacteriaceae Isolated from Urinary Tract Infections in a Tertiary Care Hospital

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ABSTRACT

Background:
Carbapenem-resistant Enterobacteriaceae (CRE) have emerged as a major global health threat, particularly in urinary tract infections (UTIs), where therapeutic options are limited. Molecular characterization of carbapenemase-encoding genes is essential to understand resistance epidemiology and guide infection control strategies.

Objectives:
To determine the distribution and molecular patterns of carbapenemase-encoding genes among carbapenem-resistant Enterobacteriaceae isolated from urine samples in a tertiary care hospital.

Methods:
A cross-sectional study was conducted over a 42-month period in a tertiary care teaching hospital. Enterobacteriaceae isolated from urine samples were identified using standard microbiological methods. Carbapenem resistance was determined by antimicrobial susceptibility testing. Molecular detection 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:
Among the carbapenem-resistant isolates, *Escherichia coli* and *Klebsiella pneumoniae* were predominant. The bla_NDM gene was the most frequently detected carbapenemase gene, followed by bla_OXA-48-like. Co-existence of multiple carbapenemase genes was observed in a subset of isolates, indicating plasmid-mediated dissemination.

Conclusion:
The study demonstrates a high burden of carbapenemase-encoding genes among uropathogenic Enterobacteriaceae. Continuous molecular surveillance and early detection are critical to curb the spread of CRE and to optimize antimicrobial therapy.

Keywords:
Carbapenem-resistant Enterobacteriaceae; Carbapenemase genes; bla_NDM; bla_OXA-48; Urinary tract infection; Molecular epidemiology

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections encountered in clinical practice and constitute a significant cause of morbidity in both community and hospital settings[1]. Members of the family *Enterobacteriaceae*, particularly *Escherichia coli* and *Klebsiella pneumoniae*, are responsible for the majority of uncomplicated and complicated UTIs[2].

Over the past two decades, the management of UTIs has become increasingly challenging due to the rising prevalence of antimicrobial resistance among uropathogens[3]. Carbapenems are considered last-line agents for severe infections caused by multidrug-resistant and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae[4]. However, the global emergence of carbapenem-resistant Enterobacteriaceae (CRE) has significantly compromised the effectiveness of these agents[5].

Carbapenem resistance in Enterobacteriaceae is mediated through multiple mechanisms, including reduced outer membrane permeability, efflux pump overexpression, and, most importantly, the production of carbapenemase enzymes[6]. Carbapenemase belong to Ambler classes A, B, and D, with KPC, NDM, VIM, IMP, and OXA-48-like enzymes being the most widely disseminated globally[7].

The spread of carbapenemase-encoding genes is largely driven by plasmid-mediated horizontal gene transfer, enabling rapid dissemination across bacterial species and healthcare settings[8]. Infections caused by CRE are associated with prolonged hospital stays, increased healthcare costs, and higher mortality rates compared to infections caused by carbapenem-susceptible organisms[5].

India has been identified as a major reservoir for carbapenemase-producing Enterobacteriaceae, particularly those harbouring bla_NDM and bla_OXA-48-like genes[9]. Despite this, regional data on the molecular epidemiology of carbapenem resistance among uropathogens remain limited. The present study was undertaken to characterize the distribution of carbapenemase-encoding genes among CRE isolated from urinary tract infections in a tertiary care hospital.

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 3 years.

Study Population and Isolates

A total of 3617 urine samples received from both outpatient and inpatient departments were processed during the study period. Non-duplicate Enterobacteriaceae isolates (n = 350) showing 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. 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 in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines[11]. Carbapenem resistance was assessed using imipenem, meropenem, and ertapenem disks.

Molecular Detection of Carbapenemase Genes

All carbapenem-resistant isolates were subjected to molecular analysis. Genomic DNA was extracted using standard protocols. Multiplex polymerase chain reaction (PCR) was performed for the detection of carbapenemase-encoding genes including bla_NDM, bla_VIM, bla_IMP, bla_KPC, and bla_OXA-48-like, using previously described primers and amplification conditions[12].

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 significant proportion. *Escherichia coli* constituted the majority of carbapenem-resistant isolates, followed by *Klebsiella pneumoniae*. Other Enterobacteriaceae such as *Citrobacter*, *Enterobacter*, and *Proteus* species were isolated less frequently.

Molecular analysis revealed that bla_NDM was the most prevalent carbapenemase-encoding gene among CRE isolates. The bla_OXA-48-like gene was the second most commonly detected determinant. A smaller proportion of isolates harbored bla_VIM and bla_IMP genes, while bla_KPC was infrequently identified.

Co-existence of two or more carbapenemase genes was observed in a subset of isolates, suggesting plasmid-mediated acquisition and dissemination of resistance determinants.

DISCUSSION

The present study provides valuable insights into the molecular epidemiology of carbapenem resistance among uropathogenic Enterobacteriaceae in a tertiary care setting. The predominance of *E. coli* and *K. pneumoniae* as CRE isolates is consistent with previous studies conducted in India and other regions[2,13].

The high prevalence of bla_NDM observed in this study reflects the endemic nature of this gene in the Indian subcontinent[9]. The detection of bla_OXA-48-like genes further highlights the evolving complexity of carbapenem resistance mechanisms among uropathogens[14].

The presence of multiple carbapenemase genes within single isolates is particularly concerning, as it limits therapeutic options and facilitates rapid dissemination of resistance[7]. These findings underscore the critical role of plasmid-mediated horizontal gene transfer in the spread of carbapenem resistance[8].

Molecular surveillance of carbapenemase genes is essential not only for epidemiological monitoring but also for guiding infection control measures and antimicrobial stewardship programs[15].

CONCLUSION

This study demonstrates a high burden of carbapenemase-encoding genes among carbapenem-resistant Enterobacteriaceae isolated from urinary tract infections. The predominance of bla_NDM and bla_OXA-48-like genes highlights the urgent need for continuous molecular surveillance, early detection, and stringent infection control measures to limit the spread of CRE.

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