# Prevalence and Characterization of Carbapenemase-producing *Escherichia coli* from a Tertiary Care Hospital in India

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## Abstract

The purpose of this study was to estimate the prevalence and to characterize the carbapenemase-producing *Escherichia coli* by various phenotypic antimicrobial susceptibility testing methods, and its performance was compared to the gold standard genotypic method. The prevalence of carbapenemase-resistant *E. coli* was found to be 65%. The phenotypic methods evaluated are cost-effective and can be used in resource-limited laboratories to rule out carbapenem resistance.

**Keywords:** Carbapenemases, carbapenem-resistant Enterobacteriaceae, *Escherichia coli,* metallo-β-lactamases

## Introduction

The high prevalence of carbapenem-resistant Enterobacteriaceae (CRE) is a growing public issue worldwide. *Escherichia coli* is one of the most common causative agents of a wide range of clinical infections varying from meningitis, urinary tract infections, and sepsis.[1] Carbapenems are the only antibiotic option left for the treatment of infections caused by multidrug-resistant *E. coli*. However, the emergence of carbapenem resistance leads to the failure of empirical therapy with carbapenems.[2,3] Our study aimed to estimate the prevalence of carbapenemase-producing *E. coli* by various phenotypic tests, and its performance was compared to genotypic-based detection method (polymerase chain reaction [PCR]).

## Microbiology Report

The study was conducted after obtaining approval from the Institute Ethics Committee (Ref No: IEC/NP-123/2011). A total of 103 clinically significant *E. coli* strains isolated various clinical specimens such as blood 7/103 (6.7%), sterile body fluids 3/103 (2.9%), endotracheal aspirate 27/103 (26.2%), pus 39/103 (37.8%), and urine 27/103 (26.2%) were screened for carbapenemase production using disk diffusion method; minimum inhibitory concentration was determination by E-test and Vitek-2 system. Phenotypic confirmation for carbapenemase production was done using Modified Hodge test, double disk synergy test (DDST), boronic acid (BA) disc test, and combined disk test (CDT). The results were interpreted based on the Clinical and Laboratory Standards Institute guidelines.[4] The presence of *bla*IMP, *bla*VIM, *bla*OXA,

**Table 1: Primers used for the detection of carbapenemase genes**

| Primer name | Sequence | Amplicon size |
|---|---|---|
| IMP-F | GGCAGTCGCCCTAAAACAAA | 737 |
| IMP-R | TAGTTACTTGGCTGTGATGG | |
| VIM-F | AAAGTTATGCCGCACTCACC | 865 |
| VIM-R | TGCAACTTCATGTTATGCCG | |
| OXA- 1-F | CGCAAATGGCACCAGCTTCAAC | 464 |
| OXA- 1-R | TCCTGCACCAGTTTTCCCATACAG | |
| KPC-F | ATGTCACTGTATCGCCGTC | 382 |
| KPC-R | AATCCCTCCGAGCGCGAGT | |
| NDM-1-F | GGTGCATGCCCGGTGAAATC | 660 |
| NDM-1-R | ATGCTGGCCTTGGGGAACG | |

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**Table 2: Results of the boronic acid disk tests for isolates with different β-lactamase contents**

| Characteristic of strain group (by PCR) | Number of isolates positive by the boronic acid test with, *n* (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | IPM | MER | ETP | FEP | CTT | FOX | CTX | CAZ |
| KPC positive (*n*=10) | 0 | 6 (60) | 5 (50) | 5 (50) | 3 (30) | 4 (40) | 3 (30) | 4 (40) |

IPM: Imipenem, MER: Meropenem, ETP: Ertapenem, FEP: Cefepime, CTT: Cefotetan, FOX: Cefoxitin, CTX: Cefotaxime, CAZ: Ceftazidime, PCR: Polymerase chain reaction

**Table 3: Prevalence of carbapenemase genes**

| Carbapenemases | Number of isolates (*n*=94) | Distribution (%) |
|---|---|---|
| KPC | 10 | 10.6 |
| NDM-1 | 58 | 61.7 |
| VIM | 29 | 30.8 |
| IMP | 2 | 2.1 |
| OXA-48 | 5 | 5.3 |

*bla*KPC, and *bla*NDM was detected by PCR using the primers described in Table 1. The PCR cycling conditions included an initial denaturation step at 94°C for 3 min and primer annealing at 56°C for 30 s, followed by 35 cycles of DNA amplification at 94°C for 30 s and elongation at 72°C for 1 min.[5] *Klebsiella pneumoniae* (ATCC BAA1705) (KPC positive) and *Enterobacter cloacae* (ATCC BAA2468) (NDM1 positive) were the control strains used in the study. Among the 103 isolates, 94 (91.26%) were suspected to have resistance to carbapenem by disk diffusion method. Disk diffusion method using ertapenem yielded the highest sensitivity of 71.2% and positive predictive value of 94.4%, making it the best test for screening. Among the other tests used for the detection of metallo-β-lactamases, CDT using meropenem–ethylenediaminetetraacetic acid showed the highest sensitivity of 58.8% and specificity of 66.6% in comparison with PCR. DDST using ceftazidime and 2- mercaptopropionic acid (MPA) showed the highest sensitivity of 87.2% and specificity of 33.3% in comparison with PCR. The phenotypic detection of KPC-possessing *E. coli* isolates was evaluated using BA disk tests using eight different β-lactam antibiotics. The results of BA disk test in comparison with PCR are depicted in Table 2. Based on the PCR assay, the most common gene detected was *bla*NDM-1, 58/94 (61.7%) in our study. The remaining isolates, 36/94 (38.2%), harbored combination of either of the genes encoding *bla*NDM-1, *bla*IMP, *bla*VIM, *bla*KPC, and *bla*OXA-46. The prevalence of various carbapenemase genes is depicted in Table 3.

## Conclusion

Our study had demonstrated a very high prevalence of carbapenemases and carbapenem resistance encoding genes in *E. coli* clinical isolates. The situation is alarming because there are very few therapeutic options available in the near future for these multidrug-resistant organisms. Combination of both phenotypic and genotypic methods would serve as a better tool for the identification of these CRE.

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### Conflicts of interest

There are no conflicts of interest.

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