

Co-occurrence of genes encoding carbapenem resistance and aminoglycoside resistance in clinical isolates of Enterobacterales

Shradha Smriti¹, Gaurav Verma¹, Sujit Pradhan², Nipa Singh¹, Subhra Snigdha Panda¹, Ipsa Mohapatra³, Dipti Pattnaik¹, Rajesh Kumar Dash¹, Liza Das¹

¹Department of Microbiology, Kalinga Institute of Medical Sciences, KIIT DU, Bhubaneswar, Odisha - India

²Department of Critical Care Medicine, Kalinga Institute of Medical Sciences, KIIT DU, Bhubaneswar, Odisha - India

³Department of Community Medicine, Kalinga Institute of Medical Sciences, KIIT DU, Bhubaneswar, Odisha - India

ABSTRACT

Introduction: This study aimed to detect the co-occurrence of carbapenem resistance genes along with aminoglycoside-modifying enzyme (AME) genes in clinical *Enterobacterales* isolates to understand the distribution of multiple resistance genes among clinical isolates.

Methods: This prospective study was conducted for six months (November 2024 to April 2025) in the department of microbiology of a tertiary care hospital. A total of 30 blood culture isolates were identified as resistant to both carbapenem and aminoglycoside antibiotics using the automated VITEK 2 compact system. The genes responsible for carbapenem resistance (*bla*_{NDM}, *bla*_{OXA-48}, *bla*_{KPC}, *bla*_{IMP}, and *bla*_{VIM}) were detected by multiplex real-time PCR, and the aminoglycoside-modifying enzyme genes [APH(3')-Ia, APH(2'')-Ib, AAC(3)-IIc, AAC(6')-Ib, and ANT(3'')-I] were detected by the conventional polymerase chain reaction method. All the clinical data, patient demographics, and molecular findings were entered in an MS Excel spreadsheet version 14.0.4734.1000 and analyzed using GraphPad/PRISM software version 10.5.0.

Results: Of the 30 *Enterobacterales* isolates, *Klebsiella pneumoniae* was the most common isolate (66.7%). Molecular detection revealed *bla*_{NDM} in 40% isolates and *bla*_{OXA48} in 10% isolates. The majority of the AME genes were in combination. The most common combination of the AME gene was AAC(6')-Ib+ AAC(3)-IIc+ ANT(3'')-I + APH(3')-I detected in 4 (13.3%) isolates. The most common combination of carbapenem and aminoglycoside resistant genes was *bla*_{NDM} + *bla*_{OXA48} + AAC(6')-Ib+ AAC(3)-IIc+ ANT(3'')-I+ APH(3')-I (13.3%). The *bla*_{OXA-48} gene had a statistically significant association with AAC(6')-Ib, ANT(3'')-I, and APH(3')-I (p < 0.05).

Conclusion: The Co-occurrence of carbapenem resistance and aminoglycoside-modifying enzyme genes in clinical *Enterobacterales* isolates limits the therapeutic option.

Keywords: Carbapenem resistance, Aminoglycoside-modifying enzymes, mCIM, eCIM, Mortality, Co-occurrence

Introduction

The increasing spread of multidrug-resistant *Enterobacterales* is a major global public health concern (1,2). Carbapenem resistance is caused either by the production of carbapenemase enzymes or by mutation in porin channels, which has led to limited treatment options (3-5). The Centers for Disease Control and Prevention (CDC) defines carbapenem resistance as non-susceptibility to at least one or more carbapenem or production of the carbapenemase enzyme (6). The Clinical and Laboratory Standards Institute

(CLSI) recommends the modified carbapenem inactivation method (mCIM), EDTA-modified version (eCIM), and Carba NP for the detection of carbapenemase enzyme production (7). Carbapenemase enzymes are produced by different carbapenemase encoding genes like: Ambler class B metallo-β-lactamases (MBLs)- *bla*_{NDM}, *bla*_{VIM}, and *bla*_{IMP}; Class A serine β-lactamases- *bla*_{KPC}, and class D oxacillinase- *bla*_{OXA-48} (8). Aminoglycoside resistance occurs primarily due to the production of aminoglycoside-modifying enzyme genes such as aminoglycoside acetyltransferase (AAC), aminoglycoside nucleotidyltransferase (ANT), and aminoglycoside phosphotransferase (APH), which is encoded by AAC, ANT, and APH genes, respectively (9-11). These resistance genes are frequently present on mobile genetic elements (MGEs) (for example, transposons, plasmids, and integrons), which promote horizontal transfer in bacterial populations (12). Few studies have indicated that clinical isolates with carbapenem resistance genes (i.e., *bla*_{NDM} and *bla*_{OXA}) have plasmids that also harbor multiple resistance genes, leading to the limitation of effective treatment options (5,13). This study aimed to evaluate the coexistence of genes

Received: July 8, 2025

Accepted: October 1, 2025

Published online: October 27, 2025

This article includes supplementary materials

Corresponding authors:

Nipa Singh

email: nipa.singh@kims.ac.in



conferring resistance to carbapenems and aminoglycosides in clinical isolates of *Enterobacterales*.

Materials and Methods

This prospective study was conducted for a period of six months from November 2024 to April 2025 in the department of microbiology of a tertiary care hospital.

Inclusion Criteria: All clinical isolates of *Enterobacterales* that were isolated from positive blood culture samples of patients admitted to the main intensive care units (ICUs).

Exclusion Criteria: All non-*Enterobacterales* isolated from positive blood culture samples, *Enterobacterales* obtained from samples other than blood, or obtained from patients admitted to ICUs other than the main ICU and wards.

Those *Enterobacterales* that were screened to be resistant to both carbapenem and aminoglycoside antibiotics phenotypically, as per CLSI 2024 guidelines, were included in the study (7). Based on the screening, 30 isolates were included, and DNA extraction of the isolates followed by molecular detection of carbapenem-resistant genes and aminoglycoside-modifying enzyme genes was done. Demographic data, clinical parameters, and outcomes of the patients were recorded and analyzed.

Sample collection and processing

Blood samples from patients diagnosed with septicemia were collected in aerobic blood culture bottles (BACT/ALERT FA and FN Plus BC bottles) under aseptic conditions. The culture bottles were incubated inside the BACT/ALERT 3D system (BioMérieux, France) until they flagged positive or upto a maximum of 5 days (14). The blood culture bottle that flagged positive was subcultured on MacConkey and blood agar plates. After incubating the plates overnight at 37°C, the isolated bacterial colony was subjected to identification and antibiotic susceptibility testing.

Identification and Antimicrobial Susceptibility Testing of Clinical Isolates

The identification and AST of the isolates were performed by an automated VITEK 2 compact system (BioMérieux, USA). All the isolates that were identified as *Enterobacterales* and were screened to be resistant to at least one carbapenem antibiotic (ertapenem, imipenem, or meropenem), as well as to any one of the aminoglycoside antibiotics (amikacin, gentamicin, tobramycin, and netilmicin), were included. The interpretation of AST was performed as per the Clinical and Laboratory Standards Institute (CLSI) 2024 M100 guidelines (7).

Phenotypic Detection of Carbapenem Resistance and Aminoglycoside Resistance

All the screened isolates resistant to both carbapenems and aminoglycosides were again phenotypically tested by the Kirby-Bauer disk diffusion method using imipenem 10 µg, ertapenem 10 µg, meropenem µg, amikacin 30 µg, gentamicin 10 µg, tobramycin 10 µg, and netilmicin 10 µg disks (HiMedia, India). *Escherichia coli* (*E. coli*) American Type Culture Collection (ATCC) 25922 was used as the quality control strain.

Phenotypic Detection of Carbapenemase Production

The modified carbapenem inactivation method (mCIM) tests were performed on all the isolates phenotypically resistant to carbapenem antibiotics as per the CLSI 2024 guidelines to detect carbapenemase-producing *Enterobacterales* (CPE) (7). For each strain tested, two tubes were prepared, each containing 2ml of Trypticase Soy Broth (TSB). One tube was EDTA-free, and the other tube was supplemented with 20µl of 0.5M EDTA. A fresh isolated colony from the test organism was inoculated into each tube using an inoculating loop.

A 10 µg meropenem disk (HiMedia, India) was dropped into each tube and incubated aerobically at 35°C for 2-4 hours. Following incubation, meropenem disks were removed from both the tubes and placed onto Mueller-Hinton Agar (MHA) plates that had been freshly inoculated with 0.5 McFarland suspension of carbapenem-sensitive *Escherichia coli* ATCC 25922 indicator strain. Next, the plates were incubated for 16-20 hours at 35 °C. The result of the mCIM test was interpreted as negative when the zone of inhibition was ≥19 mm, positive when it measured between 6 and 15mm, and also considered positive (intermediate) if small, pinpoint colonies appeared within the 16-18 mm zone of inhibition. *E. coli* ATCC 25922 was used as the quality control strain (15,16).

Molecular Detection of Antibiotic Resistance Genes

All the study isolates (n = 30), which were phenotypically confirmed to be resistant to both carbapenem and aminoglycoside antibiotics, were subsequently subjected to DNA extraction by a commercially available spin column, as per the manufacturer's procedure (TRUPCR bacterial nucleic acid extraction kit-3B BlackBio Biotech India Ltd., Bhopal, India) (17). The purity and concentration of the extracted DNA were evaluated by measuring 1 µL of the eluted DNA using a NanoDrop-Multiskan Sky spectrophotometer to measure the optical density at 260 nm and 280 nm with an absorbance ratio of ~1.8-2.0, which is considered a purity indicator of DNA samples (18).

Genes encoding carbapenem resistance (*bla*_{NDM}, *bla*_{OXA48}, *bla*_{KPC}, *bla*_{VIM}, and *bla*_{IMP}) were detected using TaqMan hydrolysis probe-based multiplex real-time polymerase chain reaction (PCR) (QuantStudio 5, Applied Biosystems, Waltham, MA, USA) by commercially available TRUPCR UTI AST Panel kit (19). The kit has a two-tube PCR assay, in tube one, primers and probe for *bla*_{KPC}, *bla*_{NDM}, and *bla*_{VIM}, and in the second tube, primers and probe of *bla*_{OXA-48} and *bla*_{IMP} were added. The assay was performed by preparing a final volume of 25 µL reaction, consisting of 20 µL Master Mix and 5 µL of DNA template.

The genes encoding aminoglycoside resistance genes [APH(3')-Ia, APH(2'')-Ib, AAC(3)-IIc, AAC(6')-Ib, and ANT(3'')-I] were detected by the conventional PCR method using previously published primers listed in supplementary Table I. The samples were amplified under different cycling conditions using a thermal cycler (Mastercycler Nexus Gradient PCR, Eppendorf, Germany) [supplementary Table II]. The thermal cycling protocol for AAC(6')-Ib, ANT(3'')-I, and APH(3')-Ia was 95°C for 5 mins, followed by 35 cycles of 1 min at 95°C, 45 sec at 55°C, 1 min at 72°C, and final extension for 5 mins at 72°C. The PCR cycling protocol for AAC(3)-IIc and APH(2'')-Ib was

95°C for 5 mins, followed by 35 cycles of 1 min at 95°C, 45 sec at 57°C, 1 min at 72°C, and final extension for 5 mins at 72°C. After amplification, 10 µL of the amplified DNA product was separated on 2% tris-Acetate Ethylene diamine tetraacetic acid (TAE) agarose gel electrophoresis and stained with ethidium bromide (final concentration: 0.5 µg/mL). After agarose gel electrophoresis, the amplified DNA band was visualized using the gel documentation system E-Box CX5 (Vilber, France). The different amplified gel products were distinguished using the molecular weight marker in the gel. Each PCR run included *Escherichia coli* ATCC 25922 as a negative control to ensure the absence of non-specific amplification.

Statistical Analysis

All the data (patient demographics, clinical parameters, and outcome) were entered into an MS Excel spreadsheet version 14.0.4734.1000. Analysis of the data was performed using the GraphPad /PRISM software version 10.5.0. A *p*-value of <0.05 was considered statistically significant.

Ethical Clearance

Clinical samples were collected after getting approval from the Institute Ethics Committee (IEC) (Ethics approval number: KIIT/KIMS/ IEC/1857/2024), with a waiver of patient consent provided due to de-identification of collected data.

Results

During the study period, 94 clinical *Enterobacterales* isolates were obtained from positive blood culture samples.

Of these, 30 isolates that were phenotypically confirmed to be resistant to both carbapenems and aminoglycosides were subjected to phenotypic and molecular detection of carbapenem and aminoglycoside resistance. Among these, *Klebsiella pneumoniae* was the most prevalent (66.7%, 20/30), followed by *Escherichia coli* (20%, 6/30), *Proteus mirabilis* (6.7%, 2/30), *Enterobacter cloacae* (3.3%, 1/30), and *Providencia rettgeri* (3.3%, 1/30). Most of the isolates were recovered from male patients (66.7%), and the most common age group was 51-60 and 61-70 years (23.3% each).

The carbapenemase production of all the 30 isolates was done by the modified carbapenem inactivation method (mCIM), and 27 (90%) tested positive, indicating that carbapenem resistance was primarily mediated by carbapenemase enzymes.

Molecular Detection of Carbapenem-Resistant Genes

Detection of carbapenem-resistant genes (*bla*_{NDM}, *bla*_{OXA48}, *bla*_{IMP}, *bla*_{VIM}, and *bla*_{KPC}) using the multiplex real-time PCR. Only *bla*_{NDM} and *bla*_{OXA-48} genes were detected, and all other genes were undetected. The real-time PCR amplification plots of *bla*_{NDM} and *bla*_{OXA48} are shown in Figure 1.

Molecular Detection of Aminoglycoside-Modifying Enzyme Genes

Five different aminoglycoside-modifying enzyme (AME) genes, APH(3')-Ia, APH(2'')-Ib, AAC(3)-IIc, AAC(6')-Ib, and ANT(3'')-I were detected in the 30 isolates using conventional PCR. Representative gel images are shown in Figures 2 (a), (b), and (c).

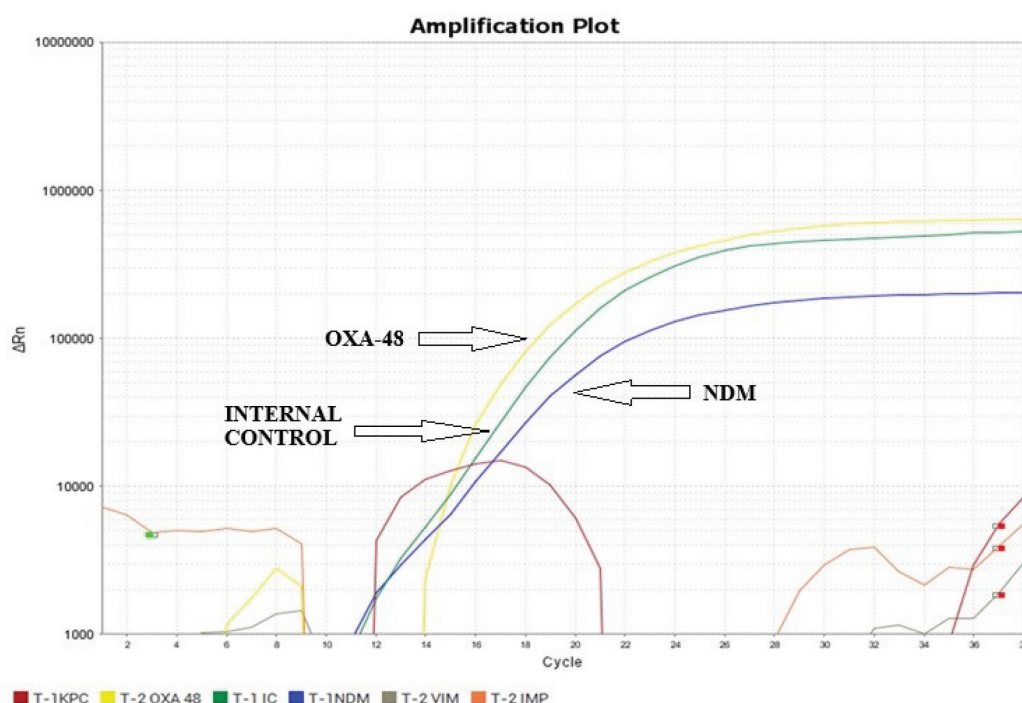


FIGURE 1 - Multiplex real-time PCR amplification plot of *bla*_{NDM} and *bla*_{OXA48}.

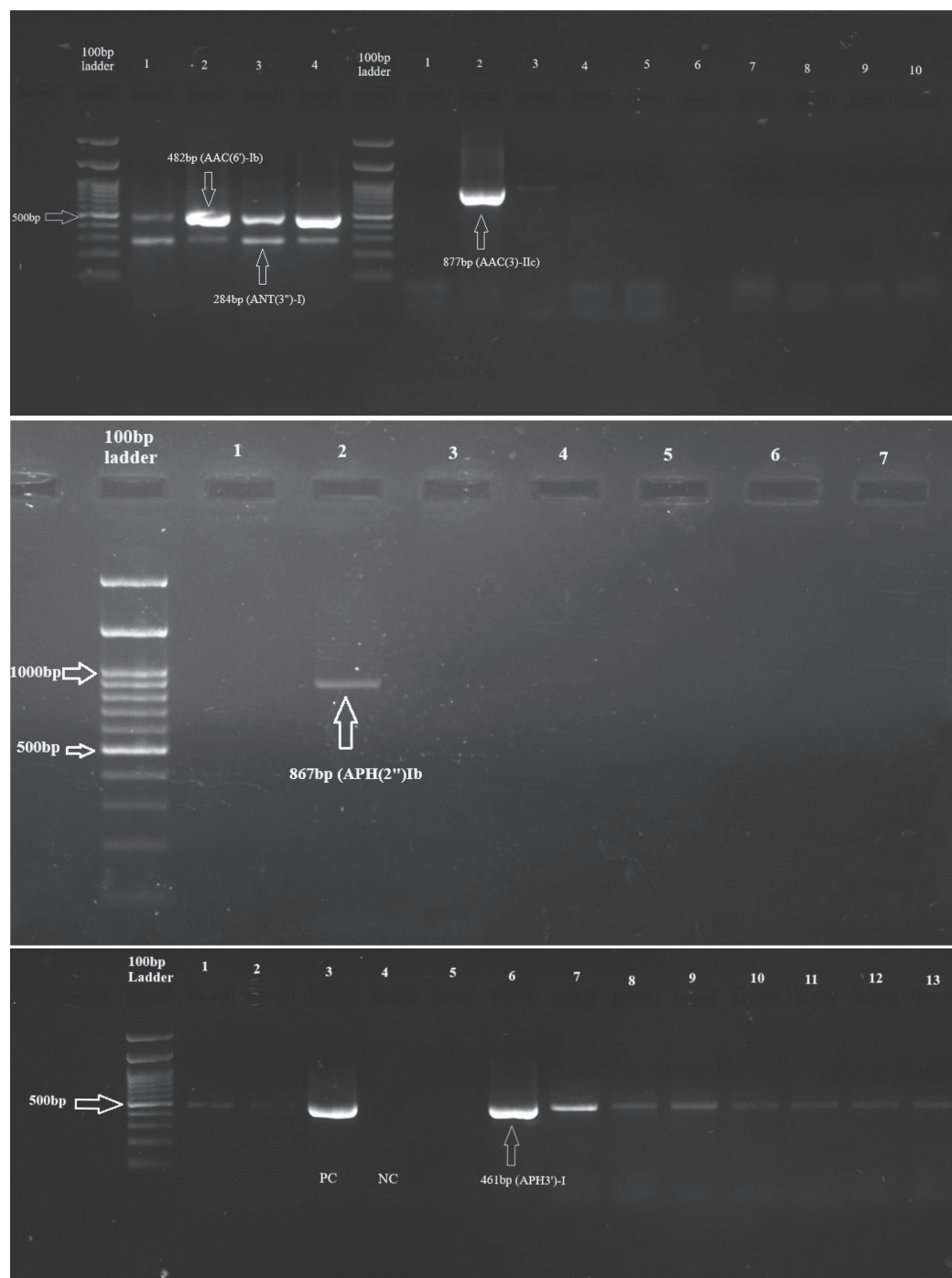


FIGURE 2 - Agarose gel image representing various amplifications of AME genes (a) multiplex PCR of AAC(6')-Ib and ANT(3'')-I, lane 6-10: AAC(3)-IIc (b) APH(2'')-Ib (c) APH(3')-I.

Detection of carbapenem resistance genes and aminoglycoside resistance genes

Among the carbapenem resistance genes, *bla*_{NDM} was detected in 12 (40%) isolates, *bla*_{OXA-48} was found in 3 (10%) isolates, and *bla*_{NDM} + *bla*_{OXA48} genes were detected in 13 (43.3%) isolates. In two bacterial isolates *bla*_{NDM} and *bla*_{OXA48} genes were undetected.

Detection of AME genes revealed AAC(6')-Ib and APH(3')-I alone were found in 2 (6.7%) isolates each. The most common combination of aminoglycoside resistance genes was AAC(6')-Ib + AAC(3)-IIc + ANT(3'')-I + APH(3')-I

detected in 4 (13.3%) isolates. No isolate harbored the ANT(3'')-I gene alone; it was present along with the other AME genes [Figure 3].

Co-occurrence of carbapenem resistance genes and aminoglycoside-resistant genes

Multiple resistance genes were found in various isolates. The most common combination of genes was *bla*_{NDM} + *bla*_{OXA48} + AAC(6')-Ib + AAC(3)-IIc + ANT(3'')-I + APH(3')-I (13.3%, 4/30), followed by *bla*_{NDM} + *bla*_{OXA48} + AAC(6')-Ib + ANT(3'')-I (10%, 3/30) of the isolates [Table 1].

Association of bla_{NDM} and bla_{OXA-48} genes with aminoglycoside-modifying enzyme genes in clinical isolates

The association between the 5 AME genes and carbapenem-resistant genes was assessed. No significant

association was observed between bla_{NDM} and any of the AME genes (p > 0.05). The bla_{OXA48} gene showed an association with AAC(6')-Ib (p = 0.044), ANT(3'')-I (p = 0.007), and APH(3')-I (p = 0.030) [Table 2].

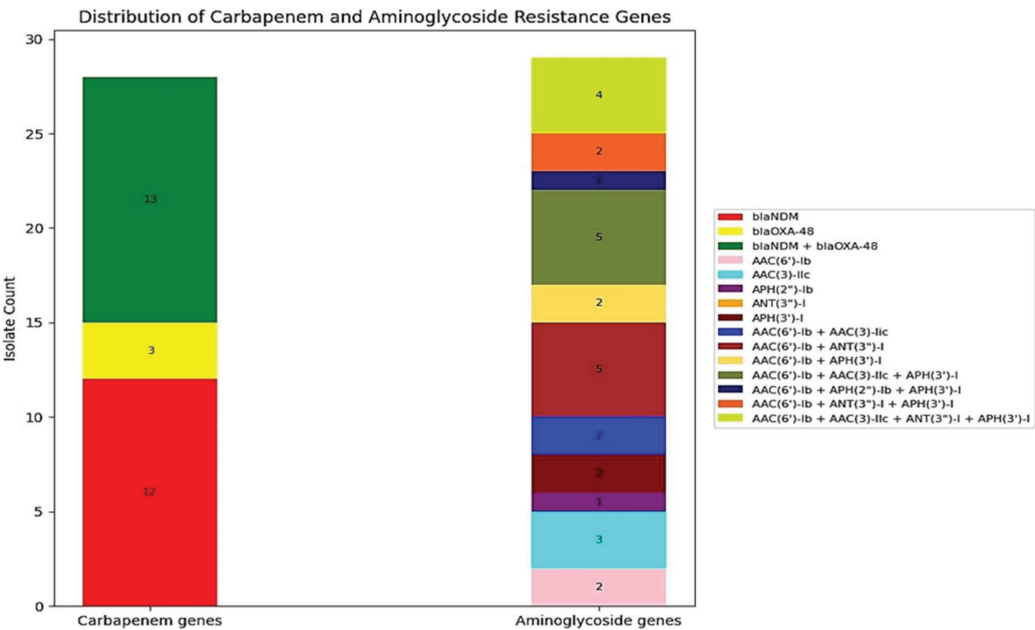


FIGURE 3 - Distribution of the carbapenem and aminoglycoside resistance genes.

TABLE 1 - Distribution of carbapenem resistance genes coexisting with aminoglycoside-modifying enzyme genes

Co-occurrence of genes mediating carbapenem and aminoglycoside resistance							Total Isolates (N = 30)	
Carbapenem resistance genes detected		Aminoglycoside-modifying enzyme genes detected					Number of isolates	Frequency (%)
blaNDM	blaOXA48	AAC(6')-Ib	AAC(3)-IIC	APH(2'')-Ib	ANT(3'')-I	APH(3')-I		
+	-	+	-	-	-	-	1	3.3%
+	-	-	+	-	-	-	3	10%
+	-	-	-	+	-	-	1	3.3%
+	-	-	-	-	-	+	1	3.3%
+	-	+	-	-	+	-	1	3.3%
+	-	+	+	-	-	-	2	6.7%
+	-	+	-	-	-	+	1	3.3%
+	-	+	+	-	-	+	2	6.7%
+	-	+	-	-	+	+	1	3.3%
-	+	-	-	-	-	+	1	3.3%
-	+	+	-	-	+	-	1	3.3%
-	+	+	+	-	-	+	1	3.3%
+	+	+	-	-	+	-	3	10%
+	+	+	-	-	+	+	2	6.7%
+	+	+	-	+	-	+	1	3.3%
+	+	+	+	-	-	+	2	6.7%
+	+	+	+	-	+	+	4	13.3%



TABLE 2 - Association of *bla*_{NDM} and *bla*_{OXA48} genes with AME genes in clinical *Enterobacteriales* isolates

Antibiotic resistance genes	AAC(6')-Ib	AAC(3)-IIc	APH(2'')-Ib	ANT(3'')-I	APH(3')-I
<i>bla</i> _{NDM} positive (N = 25)	20	12	11	2	14
<i>bla</i> _{NDM} negative (N = 5)	4	2	1	0	3
<i>p</i> -value	>0.05	0.743	0.512	0.317	0.869
<i>bla</i> _{OXA48} positive (N = 16)	15	7	10	1	12
<i>bla</i> _{OXA48} negative (N = 14)	9	7	2	1	5
<i>p</i> -value	0.044	0.732	0.922	0.007	0.03

In-hospital mortality in patients infected with multiple resistance genes

The rate of in-hospital mortality among patients infected with *Enterobacteriales* isolates harboring multiple resistance genes was 43.3%. These isolates carried both carbapenem resistance genes (*bla*_{NDM} and *bla*_{OXA48}) along with one or more aminoglycoside-modifying enzymes (AMEs) genes.

Discussion

In this study, we phenotypically detected carbapenemase production in 30 clinical *Enterobacteriales* isolates by the modified carbapenem inactivation method (mCIM). Approximately 90% of these isolates were carbapenemase producers, indicating that enzyme production plays a significant role in carbapenem resistance in *Enterobacteriales*. This is consistent with the Verma et al. and Gallego et al. studies (16, 23). Furthermore, all mCIM-positive isolates were further tested for the EDTA carbapenem inactivation method (eCIM), and all of them were metallo-β-lactamase producers.

We found that *bla*_{NDM} was the most common carbapenem resistance gene, detected in 40% of the isolates, either individually or in combination with *bla*_{OXA48}. The *bla*_{OXA48} gene was found in 10% of the isolates, while the co-occurrence of *bla*_{NDM} and *bla*_{OXA48} was detected in 43.3% of the isolates. The high prevalence of these genes is consistent with reports from other parts of India and European countries, where *bla*_{NDM} and *bla*_{OXA48} are the frequent carbapenem resistance genes found in clinical *Enterobacteriales* isolates (24-28). Several other studies have also documented the co-carriage of *bla*_{OXA48} and *bla*_{NDM} genes, which is in congruence with our findings (29-31). None of the isolates tested positive for the *bla*_{VIM}, *bla*_{KPC}, or *bla*_{IMP} genes in our study. Few Indian studies have similarly identified *bla*_{NDM} and *bla*_{OXA48} to be the most common genes detected, while *bla*_{KPC} remains infrequently detected in *Enterobacteriales* (24,25).

Among the aminoglycoside-modifying enzyme (AME) genes, both AAC(6')-Ib and APH(3')-I were detected individually in 6.7 % of the isolates. However, the coexistence of multiple AME genes was more frequent. The most common AME gene combination was AAC(6')-Ib + AAC(3)-IIc + ANT(3'')-I + APH(3')-I gene, with a positivity rate of 13.3%. The prevalence of coexisting genes has also been reported by Nie Lu et al. (32). Bacteria can acquire antimicrobial resistance genes (ARGs) through gene mutations under constant antibiotic

selection pressure. ARGs are found on mobile genetic elements (MGEs), including plasmids, transposable elements, and bacteriophages (33). The coexistence of multiple resistance genes could be attributed to IncF plasmids, which are well-known carriers of extended-spectrum β-lactamases, carbapenemase, aminoglycoside-modifying enzymes, and plasmid-mediated quinolone resistance (PMQR) genes (34). A study conducted in Spain found that the plasmid conferring aminoglycoside resistance in *Enterobacteriales* belongs to the IncF, IncFIA, or IncFIB incompatibility groups (35). Few other studies have also provided evidence suggesting that these plasmids play a crucial role in the dissemination of aminoglycoside resistance genes in drug-resistant bacteria (22).

Importantly, the study demonstrated an increased co-occurrence of carbapenem resistance genes and aminoglycoside resistance genes. The most common co-occurring resistance genes were: *bla*_{NDM} + *bla*_{OXA48} + AAC(6')-Ib + AAC(3)-IIc + ANT(3'')-I + APH(3')-I, which were detected in 13.3% of the isolates, followed by *bla*_{NDM} + *bla*_{OXA48} + AAC(6')-Ib + ANT(3'')-I, which were found in 10% of the isolates. Wangkheimayum J et al. reported a high prevalence of carbapenem and colistin resistance in aminoglycoside-resistant *Enterobacteriales*. However, they reported that most of the CRE isolates did not carry any carbapenemase genes, indicating an alternative resistance mechanism (22).

The present study investigated the association between all five aminoglycoside-modifying enzyme (AME) genes and carbapenem resistance genes. The *bla*_{OXA48} gene and three specific AME genes: AAC(6')-Ib (*p* = 0.044), ANT(3'')-I (*p* = 0.007), and APH(3')-I (*p* = 0.030) were found to have a statistically significant association. This finding could be due to the co-localization of these resistance genes on conjugative plasmids, like IncF, IncL/M, or other broad-host-range plasmids, which facilitate their horizontal transmission (36,37).

The overall in-hospital mortality was 43.3% among patients harboring multiple resistance genes (*bla*_{NDM}, *bla*_{OXA48} and at least one AME gene). This is in agreement with Baek et al., who reported a 30-day mortality rate of approximately 40.9% in CRE-infected patients harboring multiple carbapenem-resistant genes (38). A systematic review done by Falagas et al. further indicated that CRE-attributable deaths ranged from 26% to 44% (39). There are very limited studies reporting the presence of AME genes attributable to death in patients.

The presence of carbapenem resistance genes along with one or more aminoglycoside-modifying enzyme genes is a



growing concern, as it limits effective treatment options. This co-occurrence of multiple resistance genes could be due to a combination of mutation accumulation or by horizontal transfer of resistance genes via plasmids, transposons, and integrons, ultimately forming clusters of resistance genes known as “antimicrobial resistance islands” (33).

The study has certain limitations; it was conducted in a single tertiary care center with a limited sample size, so the findings cannot be fully generalizable to other regions or healthcare settings. Hence, further multi-centric studies should be conducted to confirm our findings. The sequencing of the resistant genes was not done, which would have provided further confirmation and characterization of those genes. Furthermore, we documented the co-occurrence of carbapenem resistance genes and aminoglycoside-modifying enzyme genes; however, we did not analyze the clinical outcome of the patients harboring multiple resistance genes.

Conclusion

This study highlights the increasing epidemiological risk posed by multidrug-resistant organisms with multiple resistance genes. It specifies the presence of *bla*_{NDM} and *bla*_{OXA48} in carbapenem-resistant Enterobacterales, along with circulating aminoglycoside-modifying enzyme genes in the eastern region of India. The coexistence of these genes limits the available therapeutic options, making these infections difficult to manage with an alarming mortality rate.

Acknowledgments

We would like to thank Dr. A Raj Kumar Patro, Ph.D, Consultant, Molecular Biology and Advance Diagnostics, Department of Microbiology, KIMS, for the continuous support and guidance. We would also like to acknowledge Kalinga Institute of Medical Sciences, KIIT DU, for providing financial support for carrying out this work

Disclosures

Conflict of interest: The authors declare no conflict of interest.

Financial support: It was provided by Kalinga Institute of Medical Sciences, KIIT DU, for carrying out this work.

References

- Noster J, Thelen P, Hamprecht A. Detection of multidrug-resistant Enterobacterales—from ESBLs to carbapenemases. *Antibiotics* (Basel). 2021;10(9):1140. [CrossRef](#)
- Smith HZ, Hollingshead CM, Kendall B. Carbapenem-resistant Enterobacterales. *StatPearls*. 2025. [Online](#)
- Morrill HJ, Pogue JM, Kaye KS, et al. Treatment options for carbapenem-resistant Enterobacteriaceae infections. *Open Forum Infect Dis*. 2015;2(2):ofv050. [CrossRef](#)
- Tompkins K, van Duin D. Treatment for carbapenem-resistant Enterobacterales infections: recent advances and future directions. *Eur J Clin Microbiol Infect Dis*. 2021;40(10):2053-2068. [CrossRef](#) [PubMed](#)
- Lee H, Shin J, Chung YJ, et al. Co-introduction of plasmids harbouring the carbapenemase genes, bla_{NDM}-1 and bla_{OXA}-232, increases fitness and virulence of bacterial host. *J Biomed Sci*. 2020;27(1):8. [CrossRef](#)
- Centers for Disease Control and Prevention (CDC). About Carbapenem-resistant Enterobacterales. 2024. [Online](#) (Accessed July 2025)
- Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 34th ed. CLSI supplement M100. 2024. [Online](#) (Accessed July 2025)
- Loqman S, Soraa N, Diene SM, et al. Dissemination of carbapenemases (OXA-48, NDM and VIM) producing Enterobacteriaceae isolated from the Mohamed VI University Hospital in Marrakech, Morocco. *Antibiotics* (Basel). 2021;10(5):492. [CrossRef](#) [PubMed](#)
- Zavascki AP, Klee BO, Bulitta JB. Aminoglycosides against carbapenem-resistant Enterobacteriaceae in the critically ill: the pitfalls of aminoglycoside susceptibility. *Expert Rev Anti Infect Ther*. 2017;15(6):519-526. [CrossRef](#) [PubMed](#)
- Vidal L, Gaftor-Gvili A, Borok S, et al. Efficacy and safety of aminoglycoside monotherapy: systematic review and meta-analysis of randomized controlled trials. *J Antimicrob Chemother*. 2007;60(2):247-257. [CrossRef](#) [PubMed](#)
- Shaw KJ, Rather PN, Hare RS, et al. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev*. 1993;57(1):138-163. [CrossRef](#) [PubMed](#)
- Zavascki AP, Carvalhaes CG, Picão RC, et al. Multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: resistance mechanisms and implications for therapy. *Expert Rev Anti Infect Ther*. 2010;8(1):71-93. [CrossRef](#) [PubMed](#)
- Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. *Antimicrob Agents Chemother*. 2015;59(10):5873-5884. [CrossRef](#) [PubMed](#)
- BioMérieux. BACT/ALERT® Culture Media Bottles. 2025. [Online](#) (Accessed July 2025)
- Tsai YM, Wang S, Chiu HC, et al. Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae. *BMC Microbiol*. 2020;20(1):315. [CrossRef](#) [PubMed](#)
- Verma G, Singh N, Smriti S, et al. Modified carbapenem inactivation method and ethylenediaminetetraacetic acid (EDTA)-carbapenem inactivation method for detection of carbapenemase-producing Enterobacterales and *Pseudomonas aeruginosa*. *Cureus*. 2024;16(6):e63340. [CrossRef](#) [PubMed](#)
- 3B BlackBio Dx Ltd. TRUPCR® Bacterial DNA Extraction Kit. [Online](#). (Accessed July 2025)
- Gallagher S. Quantitation of nucleic acids with absorption spectroscopy. *Curr Protoc Protein Sci*. 2001;4(Appendix):4K. [PubMed](#)
- TRUPCR Europe. TRUPCR® UTI AST Panel Kit. [Online](#) (Accessed July 2025)
- Djagbare P, Nademdega C, Andognaba UW, et al. Detection of aac3-IIC, aac(6')-Ib and armA genes encoding aminoglycoside resistance in *Klebsiella pneumoniae* in Burkina Faso. *Access Microbiology*. 2024;6(1):e000669. [CrossRef](#)
- Vakulenko SB, Mobashery S. Versatility of aminoglycosides and prospects for their future. *Clin Microbiol Rev*. 2003;16(3):430-450. [CrossRef](#) [PubMed](#)
- Wangkheimayum J, Majumder TD, Tapadar YB, et al. Occurrence of diverse aminoglycoside modifying enzymes with coexisting extended-spectrum-β-lactamases within Enterobacteriaceae isolated in India. *J Glob Antimicrob Resist*. 2020;21:369-374. [CrossRef](#) [PubMed](#)
- Gallego M, Salazar-Ospina L, Jiménez JN. The modified carbapenem inactivation method (mCIM): highly sensitive and specific tool to assess carbapenemase producing and non-producing in Gram-negative bacilli. *Hechos Microbiológicos*. 2022;13(2). [CrossRef](#)

24. Kazi M, Drego L, Nikam C, et al. Molecular characterization of carbapenem-resistant Enterobacteriaceae at a tertiary care laboratory in Mumbai. *Eur J Clin Microbiol Infect Dis*. 2015;34(3):467-472. [CrossRef PubMed](#)
25. Shanmugam P, Meenakshisundaram J, Jayaraman P. blaKPC gene detection in clinical isolates of carbapenem-resistant Enterobacteriaceae in a tertiary care hospital. *J Clin Diagn Res*. 2013;7(12):2736-2738. [PubMed](#)
26. Remya P, Shanthi M, Sekar U. Prevalence and clonal relatedness of NDM and OXA-48-producing *Klebsiella pneumoniae* in a tertiary care hospital in South India. *J Lab Physicians*. 2019;11(4):312-316. [CrossRef PubMed](#)
27. Albiger B, Glasner C, Struelens MJ, et al.; European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) working group. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. *Euro Surveill*. 2015;20(45). [CrossRef](#)
28. van Duin D, Perez F, Rudin SD, et al. Surveillance of carbapenem-resistant *Klebsiella pneumoniae*: tracking molecular epidemiology and outcomes through a regional network. *Antimicrob Agents Chemother*. 2014;58(7):4035-4041. [CrossRef PubMed](#)
29. Lascols C, Hackel M, Marshall SH, et al. Increasing prevalence and dissemination of NDM-1 metallo- β -lactamase in India: data from the SMART study (2009). *J Antimicrob Chemother*. 2011;66(9):1992-1997. [CrossRef PubMed](#)
30. Khajuria A, Praharaj AK, Kumar M, et al. Emergence of *Escherichia coli* co-producing NDM-1 and OXA-48 carbapenemases in urinary isolates at a tertiary care centre in Central India. *J Clin Diagn Res*. 2014;8(6):DC01-DC04. [CrossRef PubMed](#)
31. Das BJ, Singha KM, Wangkheimayum J, et al. Occurrence of blaOXA-48 type carbapenemase in *Escherichia coli* with coexisting resistance determinants: a report from India. *Gene Rep*. 2022;26:101459. [CrossRef](#)
32. Nie L, Lv Y, Yuan M, et al. Genetic basis of high level aminoglycoside resistance in *Acinetobacter baumannii* from Beijing, China. *Acta Pharm Sin B*. 2014;4(4):295-300. [CrossRef PubMed](#)
33. Das S, Bombaywala S, Srivastava S, et al. Genome plasticity as a paradigm of antibiotic resistance spread in ESKAPE pathogens. *Environ Sci Pollut Res Int*. 2022;29(27):40507-40519. [CrossRef PubMed](#)
34. Rozwandowicz M, Brouwer MSM, Fischer J, et al. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. *J Antimicrob Chemother*. 2018;73(5):1121-1137. [CrossRef PubMed](#)
35. Miró E, Grünbaum F, Gómez L, et al. Characterization of aminoglycoside-modifying enzymes in enterobacteriaceae clinical strains and characterization of the plasmids implicated in their diffusion. *Microb Drug Resist*. 2013;19(2):94-99. [CrossRef PubMed](#)
36. Alousi S, Salloum T, Arabaghian H, et al. Genomic characterization of multidrug-resistant *Escherichia coli* harboring blaOXA-48 on the IncL/M-type plasmid isolated from bloodstream infection. *BioMed Res Int*. 2018;2018:3036143. [CrossRef PubMed](#)
37. Sabtcheva S, Stoikov I, Georgieva S, et al. Genomic characterization of 16S rRNA methyltransferase-producing Enterobacterales reveals the emergence of *Klebsiella pneumoniae* ST6260 harboring rmtF, rmtB, blaNDM-5, blaOXA-232 and blaSFO-1 genes in a cancer hospital in Bulgaria. *Antibiotics (Basel)*. 2024;13(10):950. [CrossRef PubMed](#)
38. Baek MS, Kim JH, Park JH, et al. Comparison of mortality rates in patients with carbapenem-resistant Enterobacterales bacteraemia according to carbapenemase production: a multicenter propensity-score matched study. *Sci Rep*. 2024;14(1):597. [CrossRef PubMed](#)
39. Falagas ME, Tansarli GS, Karageorgopoulos DE, et al. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. *Emerg Infect Dis*. 2014;20(7):1170-1175. [CrossRef PubMed](#)