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Original Research Article

Molecular Characterization of Carbapenem-Resistant Enterobacterales in Clinical Isolates from a Tertiary Care SMS Hospital, Ahmedabad, Gujarat

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Abstract

Background: Carbapenem-Resistant Enterobacterales (CRE) pose a major public health challenge due to rapid dissemination, high mortality, and limited therapeutic options. Molecular detection of carbapenemase genes provides early identification and guides infection control strategies.

Aim: To determine the prevalence and molecular characteristics of carbapenemase genes among CRE isolates from a tertiary care hospital in Ahmedabad, Gujarat.

Methods: This cross-sectional observational study included clinical samples (urine, blood, sputum, pus, body fluids) received in the Microbiology Department of SMS Hospital. Enterobacterales were identified by culture, biochemical tests, and automated systems. Antimicrobial susceptibility testing (AST) was performed using CLSI guidelines. Phenotypic confirmation of carbapenem resistance was done using Carba NP and EDTA synergy tests. Molecular detection of carbapenemase genes (blaNDM, blaOXA-48, blaKPC, blaVIM, blaIMP) was carried out by PCR.

Results: Among 2210 Enterobacterales isolates, 520 (23.5%) were CRE among the 190 carbapenem-resistant Gram-negative isolates analyzed, 83.1% harbored a detectable carbapenemase gene. The most prevalent gene was NDM (33.7%), followed by co-production of NDM and OXA-48 (32.6%), and OXA-48 alone (14.2%). A small proportion showed co-existence of NDM, OXA-48, and VIM (2.1%), Klebsiella pneumoniae was the predominant CRE species. High resistance was noted to carbapenems, cephalosporins, and fluoroquinolones, while tigecycline/colistin showed the highest susceptibility.

Conclusion: NDM and OXA-48 were the most prevalent carbapenemase genes in our region. Molecular characterization provides better understandings of the genetic mechanisms of resistance and assists in implementing targeted infection control policies.

Keywords: CRE, Carbapenemase, NDM, OXA-48, PCR, Enterobacterales, Ahmedabad.

Introduction

Gram negative bacilli are the causative agents of various infections including urinary tract, bloodstream, and lower respiratory tract infections. [1] Carbapenems have long been considered the last-resort antibiotics for treating multidrug-resistant Gram-negative infections. [2]

However, the global rise of Carbapenem-Resistant Enterobacterales (CRE) has severely compromised their effectiveness. India, particularly Western India, has emerged as a hotspot for CRE, with frequent identification of NDM, KPC, OXA-48, VIM, and IMP carbapenemase genes. [3]

Ahmedabad is a major healthcare hub of Gujarat, yet limited molecular data exists on carbapenemase gene distribution in the region. Understanding local molecular epidemiology is essential for rational therapy and infection control. This study focuses on molecular characterization of CRE isolates from SMS Hospital, a tertiary care institute.

Study Design and Study Setting: This study was designed as a cross-sectional, laboratory-based investigation conducted in the Department of Microbiology at S.M.S. Hospital, Ahmedabad, a tertiary-care teaching institution catering to a large and diverse patient population. The facility receives clinical specimens from both inpatient and outpatient departments, enabling the study to capture a wide range of infectious cases. The study was carried out over a 12-month period, allowing sufficient time for the collection and detailed analysis of Enterobacterales isolates exhibiting carbapenem resistance.

Study Population and Sample Collection: Clinical samples were received as part of routine diagnostic services and included a wide variety of specimen types such as urine, blood, sputum, pus and wound swabs, endotracheal aspirates, and sterile body fluids (including cerebrospinal, pleural, peritoneal, and synovial fluids). Each specimen was processed according to standard microbiological practices to ensure optimal recovery of pathogens. Only Enterobacterales isolates demonstrating phenotypic resistance to at least one carbapenem were included in the study. Duplicate isolates from the same patient and environmental contaminants were excluded to avoid skewing the results.

Inclusion Criteria

- Clinical isolates belonging to the family Enterobacterales obtained from patient specimens.
- Isolates showing reduced susceptibility or resistance to any carbapenem (imipenem, meropenem, or ertapenem) as per CLSI interpretive criteria.

Exclusion Criteria

- Non-Enterobacterales isolates.
- Duplicate isolates from the same patient.
- Isolates lacking phenotypic evidence of carbapenem resistance.

Identification of Bacterial Isolates: All isolates were initially cultured on Blood agar and MacConkey agar plates and incubated at 37°C for 18–24 hours. Colony morphology, lactose fermentation characteristics, and pigment production were recorded.

Gram staining was performed to confirm gram-negative bacilli morphology. Preliminary identification was carried out using conventional biochemical tests such as oxidase, indole, citrate utilization, triple sugar iron (TSI) reactions, and urease, motility, and carbohydrate fermentation profiles.

Wherever available, automated identification systems (e.g., VITEK 2 Compact or similar platforms) were employed to ensure accuracy and rapid identification.

Antimicrobial Susceptibility Testing (AST): Antimicrobial susceptibility testing of all isolates was performed using either the Kirby–Bauer disk diffusion method or an automated AST system, following Clinical and Laboratory Standards Institute (CLSI) guidelines. Carbapenem antibiotics tested included imipenem, meropenem, and ertapenem. The results were interpreted using CLSI breakpoints. Quality control strains such as Escherichia coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603 were included during each batch of testing.

Molecular Detection of Carbapenemase Genes

DNA Extraction: Genomic DNA was extracted using a heat-lysis (boiling) method or a commercial DNA extraction kit, depending on availability. Extracted DNA was stored at –20°C until further use.

Polymerase Chain Reaction (PCR): PCR was performed to detect the presence of common carbapenemase-encoding genes, including blaNDM, blaOXA-48, blaKPC, blaVIM, and blaIMP. Each gene was amplified using published primer sets with standardized PCR cycling conditions. Amplicon sizes were compared against molecular weight markers on agarose gel electrophoresis to confirm gene presence.

Positive control strains for each gene were included whenever available, and nuclease-free water served as a negative control.

Statistical Analysis: All laboratory results and patient data were recorded in Microsoft Excel spreadsheets and subsequently analyzed using SPSS software. Descriptive statistics such as frequencies, percentages, and mean values were calculated.

Results

Out of the total 8532 samples processed, gram-negative bacilli were isolated in 2210 samples. This included Klebsiella pneumoniae (810), Escherichia coli (920), Pseudomonas aeruginosa (335), Acinetobacter baumannii (80), Proteus spp. (55), Salmonella (7), Citrobacter spp. (3) and Enterobacter spp. (7). Out of the 2,351 isolates, 520 were Carbapenem resistant with a prevalence of 26.5% in the hospital.

Table 1: Distribution of Gram-Negative Bacilli (GNB) Isolated From Clinical Samples (n = 2210)

Organism	Number of Isolates (n)	Percentage (%)
Escherichia coli	920	41.6%
Klebsiella pneumoniae	810	36.6%
Pseudomonas aeruginosa	335	15.2%
Acinetobacter baumannii	80	3.6%
Proteus spp.	55	2.5%
Salmonella spp.	7	0.3%
Citrobacter spp.	3	0.1%
Enterobacter spp.	7	0.3%
Total GNB Isolates	**2210**	**100%**

Out of 520 CRO, Klebsiella pneumoniae (41.5 %) was most commonly isolated followed by Escherichia coli (36.5%), Pseudomonas aeruginosa (15.1%), Acinetobacter baumannii (3.6%) and Enterobacter species (0.42%)

Table 2: Distribution of Carbapenem-Resistant Organisms (CRO) Among Gram-Negative Bacilli (n = 520)

Organism	Percentage (%)	Number of Isolates (n)
Klebsiella pneumoniae	41.5%	216
Escherichia coli	36.5%	190
Pseudomonas aeruginosa	15.1%	78
Acinetobacter baumannii	3.6%	19
Enterobacter spp.	0.42%	2
Total	**100%**	**520**

CRO was isolated most commonly from urine samples (148 isolates, 28.4%), followed by blood (123 isolates, 23.7%), endotracheal secretions (96 isolates, 18.4%), tissue/pus samples (71 isolates, 13.7%), sputum (44 isolates, 8.4%), and sterile body fluids (38 isolates, 7.4%) in this study.

Table 3: Distribution of Carbapenem-Resistant Organisms (CRO) According to Sample Type (n = 520)

Sample Type	Number of CRO Isolates (n)	Percentage (%)
Urine	148	28.4%
Blood	123	23.7%
Endotracheal secretions	96	18.4%
Tissue / Pus	71	13.7%
Sputum	44	8.4%
Sterile body fluids	38	7.4%
Total	**520**	**100%**

The majority of the CRO isolates were recovered from ICUs (300 isolates, 57.7%), followed by hospital wards (201 isolates, 38.6%), while only 19 isolates (3.68%) were obtained from outpatient departments (community settings).

Table 4: Distribution of Carbapenem-Resistant Genes Among Different Gram-Negative Bacilli (n = 190)

Gene / Species	Escherichia coli	Enterobacter spp.	Klebsiella pneumoniae	Pseudomonas aeruginosa	Acinetobacter baumannii	Total
NDM (n = 64)	9	1	43	4	7	64
OXA-48 (n = 27)	6	0	19	2	0	27
NDM + OXA-48 (n = 62)	9	0	53	0	0	62
NDM + OXA-48 + VIM (n = 4)	1	0	2	1	0	4
IMP (n = 0)	0	0	0	0	0	0
VIM (n = 1)	0	0	0	1	0	1
KPC (n = 0)	0	0	0	0	0	0
NONE (n = 32)	6	1	17	5	3	32

Discussion

In the present study, 520 out of 2,210 Gram-negative bacilli (GNB) were carbapenem-resistant, giving an overall carbapenem-resistance prevalence of 26.5%.

This rate is considerably higher than the results reported in a in which the prevalence of carbapenem-resistant GNB was only 4.2%, despite an overall GNB prevalence of 37.9%. [4] The comparison clearly suggests a progressive rise in carbapenem resistance over the years, reflecting the increasing antimicrobial pressure and the spread of resistant clones in healthcare settings. In our study, carbapenem resistance was most common in Klebsiella pneumoniae (59.9%), followed by Escherichia coli (20.18%), with smaller proportions among non-fermenters (Pseudomonas aeruginosa and Acinetobacter baumannii). However, this contrasts with multiple studies from Southeast Asia, where carbapenem-resistant non-fermenters predominate, and CRE constitute a smaller proportion. [5] One such study reports that 82.3% of all CROs were A. baumannii or P. aeruginosa, while only 17.7% were K. pneumoniae or E. coli. [6] Similarly, Cai et al. observed carbapenem resistance in 45% of A. baumannii and 19% of P. aeruginosa,

but only 1% of Enterobacteriaceae. [4] Thus, our finding of a higher proportion of CRE compared to CR non-fermenters indicates a shift in epidemiology in our hospital.

We observed 0.32% carbapenem resistance in Enterobacter species, unlike a study from a tertiary-care centre in Mumbai, where Enterobacter spp. showed no carbapenem resistance. Similarly, no carbapenem resistance was seen in Salmonella spp., consistent with earlier literature stating that CR in Salmonella is exceedingly rare. [6]

Site-specific distribution of CRO showed that resistance varied with sample type. In our dataset of 520 CRE, the majority were isolated from urine (28.4%), followed by blood (23.7%), endotracheal secretions (18.4%), tissue/pus (13.7%), sputum (8.4%), and sterile body fluids (7.4%). Other studies have reported higher resistance in urine and pus samples, with relatively lower rates in blood isolates. [6,7]

A significant proportion of isolates (57.7%) were from ICUs, followed by wards (38.6%), with only 3.68% from OPDs, which aligns with other studies showing a similar trend of CR predominance in critical-care areas. 6,7 A multicentric Indian study also documents that carbapenem resistance is primarily associated with healthcare-associated infections. [8] However, detection of CRO in outpatient samples, though low, indicates emergence of community-onset carbapenem resistance, a developing public-health concern. Since asymptomatic carriage can contribute to CRE transmission in hospitals, routine screening of high-risk patients may be warranted. [9] Richter et al. recommend developing hospital-specific screening policies based on local epidemiology and resources. [10]

Among the 520 CRE isolates, 83.15% carried a carbapenemase gene. The most prevalent was NDM, detected in 33.68%, aligning with earlier studies. [11-13] In contrast to several studies that reported absence of VIM, we detected 0.5% VIM and 2.14% co-existence of NDM, OXA-48, and VIM. [14-17] KPC was not detected in any isolate, whereas in parts of South Asia, KPC is the dominant carbapenemase in K. pneumoniae. [18,19] Although IMP-type enzymes are frequently associated with Acinetobacter and Pseudomonas, we did not detect IMP in our isolates. [20,21] The prevalence of OXA-48 differed significantly from some published studies. While OXA-48 is reported as the most common carbapenemase in E. coli and absent in non-fermenters in certain regions, [18] we observed co-existence of NDM and OXA-48 in 32.6% of isolates—much higher than a South Indian study (12.5%). [19] Another study from Mumbai reported higher carbapenem MICs (>32 μg/mL) in dual carbapenemase producers, which has therapeutic implications, especially in settings where colistin–carbapenem combinations are frequently used. [20]

No carbapenemase gene was detected in 16.8% of isolates, suggesting alternative resistance mechanisms, such as porin loss or efflux pumps. This proportion is lower than that reported in another study, where 30% of CR isolates lacked detectable carbapenemase genes. [21]

Differences in the molecular epidemiology of carbapenem resistance are likely due to geographical variation, antibiotic prescribing patterns, infection-control practices, and different study time periods. While most surveillance efforts focus on CRE, our findings indicate a growing burden of carbapenem resistance among non-fermenting GNB as well. Limited access to molecular testing in many regions also leads to incomplete data. [7,8] Overall, available evidence shows that carbapenem resistance patterns evolve over time and vary across regions, underscoring the importance of ongoing monitoring of carbapenemase genes to guide therapy and control CRO transmission.

Conclusion

CRE isolates in SMS Hospital demonstrated a high genetic diversity of carbapenemase enzymes, with NDM and OXA-48 emerging as the predominant resistance determinants. Molecular characterization of these isolates provides critical insights into the local epidemiology of carbapenem resistance and is essential for guiding effective infection control strategies as well as optimizing antimicrobial therapy. Based on these findings, it is recommended that the hospital implement routine PCR-based screening, particularly for high-risk specimens and patients, in order to enable early detection of CRE carriers. Strengthening infection control practices—including isolation protocols, hand hygiene compliance, and environmental decontamination—is also necessary to limit transmission within healthcare settings. Additionally, reinforcing antibiotic stewardship programs will help ensure the judicious use of carbapenems and other broad-spectrum antibiotics, thereby reducing selective pressure and slowing the emergence of further resistance.

References

1. Alizadeh N, Rezaee M, Kafil H, Barhaghi M, Memar M, Milani M. Detection of carbapenem-resistant Enterobacteriaceae by chromogenic screening media. J Microbiol Methods. 2018;153:40-4
2. Lutgring J, Limbago B. The problem of carbapenemase-producing-carbapenem-resistant-Enterobacteriaceae detection. J Clin Microbiol. 2016; 54(3):529-34.

3. Lutgring J. Carbapenem-resistant Enteroba cteriaceae: an emerging bacterial threat. Semin Diagn Pathol. 2019;36(3):182-6
4. Cai B, Echols R, Magee G, Ferreira J, Morgan G, Ariyasu M. Prevalence of Carbapenem-Resistant Gram-Negative Infections in the United States Predominated by Acinetobacter baumannii and Pseudomonas aeruginosa
5. Hsu L, Apisarnthanarak A, Khan E, Suwant arat N, Ghafur A, Tambyah P. Carbapenem-Resistant Acinetobacter baumannii and Enterobacteriaceae in South and Southeast Asia. Clin Microbiol Rev. 2017;30(1):1-22.
6. Verma S, TS, Bisure K. Prevalence of Carbapenem resistant Enterobacteriaceae-a study in Tertiary Care Hospital in Mumbai. J Evol Med Dent Sci. 2018;7(45):4909-12.
7. Parimala T. Screening of carbapenem resistant enterobacteriaceae among nosocomial isolates: a study from south India. Indian J Curr Microb App Sci. 2017;6(4):460-5.
8. Mathur P, Malpied P, Walia M, Srikantiah P, Gupta S, Lohiya A. Health-care-associated bloodstream and urinary tract infections in a network of hospitals in India: a multicenter, hospital-based, prospective surveillance study. Lancet Global Health. 2022;10(9): e131 7-25.
9. Fadlallah M, Salman A, Salem-Sokhn E. Updates on the Status of Carbapenem-Resistant Enterobacterales in Lebanon. Int J Microbiol. 2023.
10. Richter S, Marchaim D. Screening for carbapenem-resistant Enterobacteriaceae: Who, When, and How? Virulence. 2017; 8(4):417-26.
11. Mohan B, Hallur V, Singh G, Sandhu H, Appannanavar S, Taneja N. Occurrence of blaNDM-1 & absence of blaKPC genes encoding carbapenem resistance in uropathogens from a tertiary care Centre from North India. Indian J Med Res. 2015; 142(3):336-43.
12. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D. Dissemination of Carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among Carbapenem-Resistant Enterobacteriaceae Isolated From Adult and Children Patients in China. Front Cell Infect Microbiol. 2020;10.
13. Elrahem AA, El-Mashad N, Elshaer M, Ramadan H, Damiani G, Bahgat M. Carbapenem Resistance in Gram-Negative Bacteria: A Hospital-Based Study in Egypt. Medicina (Kaunas). 2023;59(2)
14. Garg A, Garg J, Kumar S, Bhattacharya A, Agarwal S, Upadhyay G. Molecular epidemiology & therapeutic options of carbapenem-resistant Gram-negative bacteria. Indian J Med Res. 2019;149(2):285-9.
15. Mohan B, Hallur V, Singh G, Sandhu H, Appannanavar S, Taneja N. Occurrence of blaNDM-1 & absence of blaKPC genes encoding carbapenem resistance in uropathog ens from a tertiary care Centre from North India. Indian J Med Res. 2015;142(3):336-43
16. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D. Dissemination of Carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among Carbapenem-Resistant Enterobacteriaceae Isolated From Adult and Children Patients in China. Front Cell Infect Microbiol. 2020;10.
17. Elrahem AA, El-Mashad N, Elshaer M, Ramadan H, Damiani G, Bahgat M. Carbapenem Resistance in Gram-Negative Bacteria: A Hospital-Based Study in Egypt. Medicina (Kaunas). 2023;59(2).
18. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D. Dissemination of Carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among Carbapenem-Resistant Enterobacteriaceae Isolated From Adult and Children Patients in China. Front Cell Infect Microbiol. 2020;10.
19. Wang Q, Wang X, Wang J, Ouyang P, Jin C, Wang R. Phenotypic and Genotypic Characterization of Carbapenem-resistant Enterobacteriaceae: Data From a Longitudinal Large-scale CRE Study in China (2012-2016). Clin Infect Dis. 2012;67(suppl_2):196-205
20. Anandan S, Damodaran S, Gopi R, Bakthavatchalam YD, Veeraraghavan B. Rapid Screening for Carbapenem Resistant Organisms: Current Results and Future Approaches. J Clin Diagn Res. 2015;9(9).
21. Elrahem AA, El-Mashad N, Elshaer M, Ramadan H, Damiani G, Bahgat M. Carbapenem Resistance in Gram-Negative Bacteria: A Hospital-Based Study in Egypt. Medicina (Kaunas). 2023;59(2).