ORIGINAL ARTICLE

# Identification of carbapenemase genes and evaluation of in vitro activity of ceftazidime-avibactam against carbapenem-resistant *Enterobacterales*

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## ABSTRACT

**Background:** Infections caused by carbapenem-resistant *Enterobacterales* are becoming an intensifying global concern because of the scarce treatment options available. Carbapenem resistance plays a major role in increasing morbidity and mortality rates in healthcare settings. Ceftazidime-avibactam (CZA), demonstrates in vitro effectiveness against multi-drug resistant (MDR) organisms. The aim of the study was to identify various types of carbapenemase genes present among carbapenem-resistant *Enterobacterales* (CRE) and assess the in-vitro activity of ceftazidime-avibactam against carbapenem-resistant *Enterobacterales*.

**Material and Methods:** This prospective study was carried out in the Microbiology section of the Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, from January to May 2024. Carbapenem-resistant *Enterobacterales* (CRE) isolated from blood cultures of patients were included in the study. The detection of carbapenem-resistant genes was conducted using the Xpert Carba-R assay. Antimicrobial susceptibility testing was performed using the Sensititre automated system.

**Results:** During this period, sixty CRE isolates, recovered from blood culture samples were analysed. The most prevalent gene detected was NDM, found in 43 isolates (71.66%), followed by a combination of NDM and OXA-48 in 13 isolates (21.66%), and OXA-48 alone in 4 isolates (6.66%). Ceftazidime-avibactam (CZA) exhibited susceptibility in only 4 isolates (6.66%). All four of these isolates carried the OXA-48 gene alone.

**Conclusion:** The study's findings indicated that, within the timeframe investigated, the NDM gene was the predominant resistant gene among CRE isolates, accounting for 93.33% of cases. Ceftazidime-avibactam demonstrated good activity against CRE isolates carrying the OXA-48 gene alone. However, it was not active against CRE isolates containing the NDM gene.

**Key Words:** Carbapenem-resistant *Enterobacterales*, Carbapenemases, Ceftazidime-avibactam

## BACKGROUND

Carbapenem-resistant *Enterobacterales* (CRE) present a major threat to global public health. *Enterobacterales* that exhibit resistance to at least one carbapenem in vitro are referred to as CRE by the Centers for Disease Control and Prevention (CDC).[1] Treating CRE infections can pose significant challenges and may result in morbidity and mortality, particularly among immunocompromised individuals or within healthcare environments where transmission is more likely to happen. The statistics from the CDC's publication highlight the seriousness of the issue, with more than 1,000 deaths annually attributed to CRE infections in the United States alone.[2]

*Enterobacterales* develop resistance to carbapenems through three primary mechanisms: the production of carbapenemase, efflux pumps, and mutations in porins. Among these, carbapenemase production stands out as the predominant mechanism of resistance. *Enterobacterales* that carry carbapenemase, enabling them to resist carbapenems, are referred to as carbapenemase-producing *Enterobacterales* (CPE). Carbapenemases are a class of β-lactamases recognized for their broad hydrolytic activity. They are capable of degrading penicillins, cephalosporins, monobactams, and carbapenems. Bacteria that produce these β-lactamases can lead to severe infections, as the carbapenemase activity makes numerous β-lactam antibiotics ineffective against them.[3] According to the Ambler classification system, β-lactamases are divided into three classes. Class A includes enzymes such as

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*Klebsiella pneumoniae* carbapenemases (KPC), Class B consists of metallo-β-lactamases (MBLs) like New Delhi metallo-β-lactamase (NDM), and Class D contains OXA-48 carbapenemase. While Class A and Class D carbapenemases use serine at their active sites, Class B carbapenemases require zinc for the hydrolysis of β-lactams.[4]

Polymyxin antibiotics, such as colistin, have traditionally been used to treat resistant Gram-negative bacteria, including CRE isolates. However, there is increasing concern over the emergence of resistance to these medications.[5] Moreover, polymyxins are associated with significant nephrotoxicity, leading to their exclusion from current treatment recommendations for CRE by the Infectious Diseases Society of America (IDSA).[6]

Aminoglycosides such as amikacin are protein synthesis inhibitors which gained importance because of their ability to retain in vitro activity against CREs and they can also be synergic to other classes of antibiotics such as polymyxins.[7]

Fosfomycin, an antibiotic identified in 1969, impede the synthesis of cell walls in both Gram-positive and Gram-negative bacteria, such as *Enterobacterales*, and remains effective against certain CRE strains. While historically administered orally for lower urinary tract infections, there's now increasing attention on its intravenous application for multidrug-resistant organisms such as CREs.[8]

Ceftazidime-avibactam (CZA) is a novel combination of a beta-lactam antibiotic (Ceftazidime) and a beta-lactamase inhibitor (Avibactam). It exhibits in vitro effectiveness against multi-drug resistant (MDR) organisms, including those producing extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases, OXA-48 carbapenemases, and *Klebsiella pneumoniae* carbapenemases (KPCs). Additionally, it provides coverage against MDR Pseudomonas spp. However, CZA is not active against metallo-beta-lactamases (MBLs) such as NDM.[9, 10]

Ceftazidime-avibactam (CZA) was granted FDA approval in 2015, for treating complicated intra-abdominal infections in combination with metronidazole. It was also approved for treating complicated urinary tract infections, including pyelonephritis, caused by multidrug-resistant gram-negative pathogens.[1]

In Pakistan, Ceftazidime-avibactam has become available recently. However, there's a dearth of information regarding its efficacy against carbapenem-resistant *Enterobacterales* (CRE). This lack of data is due to limited local prevalence information on CRE genes and the absence of diagnostic tools to identify these genes. The current study sought to examine how common different carbapenem-resistance genes are among carbapenem-resistant *Enterobacteriaceae* (CRE) and to evaluate the effectiveness of Ceftazidime-avibactam against these CRE strains in vitro.

## MATERIAL AND METHODS

This prospective study was carried out in the microbiology section of Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC), Lahore from January 2024 to May 2024 after seeking approval from the Institutional Review Board Committee (vide reference number IRB-22-36).

The sample size of sixty was calculated using a 95% confidence level and a 4% margin of error, based on an anticipated susceptibility rate of 97.5% for ceftazidime-avibactam among CRE.[11] The study included CRE isolates recovered from blood culture specimens. Isolates that were susceptible to carbapenems and CREs isolated from sites other than blood were excluded from the study.

These blood culture samples were originally incubated in an automated blood culture system, BACT/ALERT VIRTUO by BioMérieux, France. Once flagged as positive, these samples were subcultured on to chocolate, blood, and MacConkey agar plates. The identification of isolates was confirmed using API 20E kits by BioMérieux, France. *Enterobacterales* that were reported as resistant to all carbapenems was included in the study. Duplicates isolates were excluded. Isolates were randomly selected through consecutive sampling.

Further testing was done via Kirby-Bauer disk diffusion methodology against ceftazidime-avibactam (50µg), amikacin (30µg), and fosfomycin (200µg) on Muller–Hinton agar. Disk elution testing for colistin (10µg) was also performed. These were in accordance with the Clinical Laboratory Standards Institute (CLSI) M100 guidelines, 34th edition (2024).[12]

The detection of carbapenem-resistant genes was conducted through Xpert Carba-R by BioMérieux, France, utilizing real-time PCR methodology. This test

targeted genes including KPC, NDM, OXA-48, VIM, and IMP. Broth microdilution for the above antibiotics was also done through the Sensititre plates (EUMDRXXF). These were inoculated using the Thermo Scientific Sensititre automated antimicrobial susceptibility testing (AST) system and incubated at 37ºC for 24 hours. Subsequently, the isolates were logged into the SWIN Software System, and the Sensititre plates were read using the Vizion Digital MIC Viewing System following overnight incubation. The Minimum inhibitory concentrations (MICs) of ceftazidime-avibactam, colistin, amikacin and fosfomycin were recorded against each CRE isolate in accordance with the guidelines provided by CLSI M100 34th edition (2024).[12] The fosfomycin interpretive breakpoints specified by CLSI M100 34th edition, 2024 for urinary *Escherichia coli* isolates were extrapolated and applied to other *Enterobacterales*.

The Statistical Package for Social Sciences (SPSS) version 24.0 by IBM, Armonk, NY, was employed for compiling and analyzing both clinical and microbiological data. Descriptive data were presented using frequencies and percentages. The frequencies of carbapenem-resistant genes and susceptibilities of ceftazidime-avibactam, colistin, amikacin and fosfomycin against CRE isolates were depicted through percentages and graphical representations.

## RESULTS

Sixty CRE isolates were included in this study. *Escherichia coli* made up the largest bulk with 38 isolates, accounting for 63.33% of them. Following this, *Klebsiella pneumoniae* represented 28.33% (17 isolates), *Citrobacter koseri* 5% (3 isolates), and *Enterobacter cloacae* 3.33% (2 isolates). Among these isolates, 35 (58.33%) were from male patients and 25 (41.66%) from female patients. The most prevalent gene detected was NDM, found in 43 isolates (71.66%), followed by a combination of NDM and OXA-48 in 13 isolates (21.66%), and the OXA-48 alone in 4 isolates (6.66%) (Figure 1). Hence, a significant proportion of 56 CRE isolates (93.33%) were recognized as carrying the NDM gene, either alone or in combination with the OXA-48 gene.

Ceftazidime-avibactam (CZA) exhibited susceptibility in only 4 isolates (6.66%). All four of these isolates carried the OXA-48 gene. Out of 55 isolates resistant to CZA, 43 isolates (76.78%) carried only the NDM gene whereas, and 13 (23.21%) harbored both NDM and OXA-48 genes (Table 1). There were no inconsistencies between the results obtained from disk diffusion testing and MIC testing of CZA across the isolates.

Both the disk elution method and the Sensititre automated AST system were utilized to assess colistin susceptibility against CREs. Out of the samples tested, five isolates (8.33%) were found resistant to colistin according to both methods, while 55 isolates (91.66%) demonstrated intermediate susceptibility. There was no discordance between the results from both methods. All of the five colistin-resistant isolates, were identified as *Klebsiella pneumoniae*. Out of the 55 isolates exhibiting intermediate susceptibility to colistin, 41 (74.54%) carried only the NDM gene, 10 (18.18%) harbored both NDM and OXA-48 genes, and 4 (7.27%) had only the OXA-48 gene. Among these, three isolates (60%) were positive for both NDM and OXA-48 genes, while the remaining two isolates (40%) showed detection of the NDM gene alone. The prevalence of colistin resistance among *Klebsiella pneumoniae* CRE isolates was determined to be 29.41%, with 5 out of 17 isolates displaying resistance. The highest recorded colistin MIC was 16 µg/ml.

33 isolates (55%) were found to be susceptible to amikacin, while 27 isolates (45%) were resistant. No discrepancies were observed between the results obtained from the disk diffusion testing and MIC testing conducted via Sensititre automated AST methods. Out of 33 isolates susceptible to amikacin, 29 (87.87%) contained only the NDM gene, while 4 (12.12%) harbored both NDM and OXA-48 genes. Among the 27 isolates resistant to amikacin, 14 (51.85%) had only the NDM gene, 9 (33.33%) possessed both NDM and OXA-48 genes, and 4 (14.81%) possessed the OXA-48 gene alone.

The majority of 44 isolates (73.33%), showed susceptibility to fosfomycin (Table 1). There were no inconsistencies between the results of disk diffusion testing and MIC testing. Out of 44 isolates susceptible to fosfomycin, 33 (75%) contained only the NDM gene, 10 (22.72%) harbored both NDM and OXA-48 genes, and 1 (2.27%) contained the OXA-48 gene alone. Among the 16 isolates resistant to fosfomycin, 10 (62.5%) possessed the NDM gene, 3 (18.75%) harbored

both NDM and OXA-48 genes, and 3 (18.75%) had only the OXA-48 gene.

**Table-I: Carbapenem-resistant genes and the susceptibilities of antimicrobials (n=60).**

| | | Antimicrobials | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Colistin | | CZA | | Fosfomycin | | Amikacin | |
| | | I | R | S | R | S | R | S | R |
| Resistance genes | NDM | 41 (74.54%) | 2 (40%) | - | 43 (76.78%) | 33 (75%) | 10 (62.5%) | 29 (87.87%) | 14 (51.85%) |
| | OXA-48 | 4 (7.27%) | - | 4 (6.66%) | - | 1 (2.27%) | 3 (18.75%) | - | 4 (14.81%) |
| | NDM + OXA-48 | 10 (18.18%) | 3 (60%) | - | 13 (23.21%) | 10 (22.72%) | 3 (18.75%) | 4 (12.12%) | 9 (33.33%) |
| | **Total** | **55** | **5** | **4** | **56** | **44** | **16** | **33** | **27** |

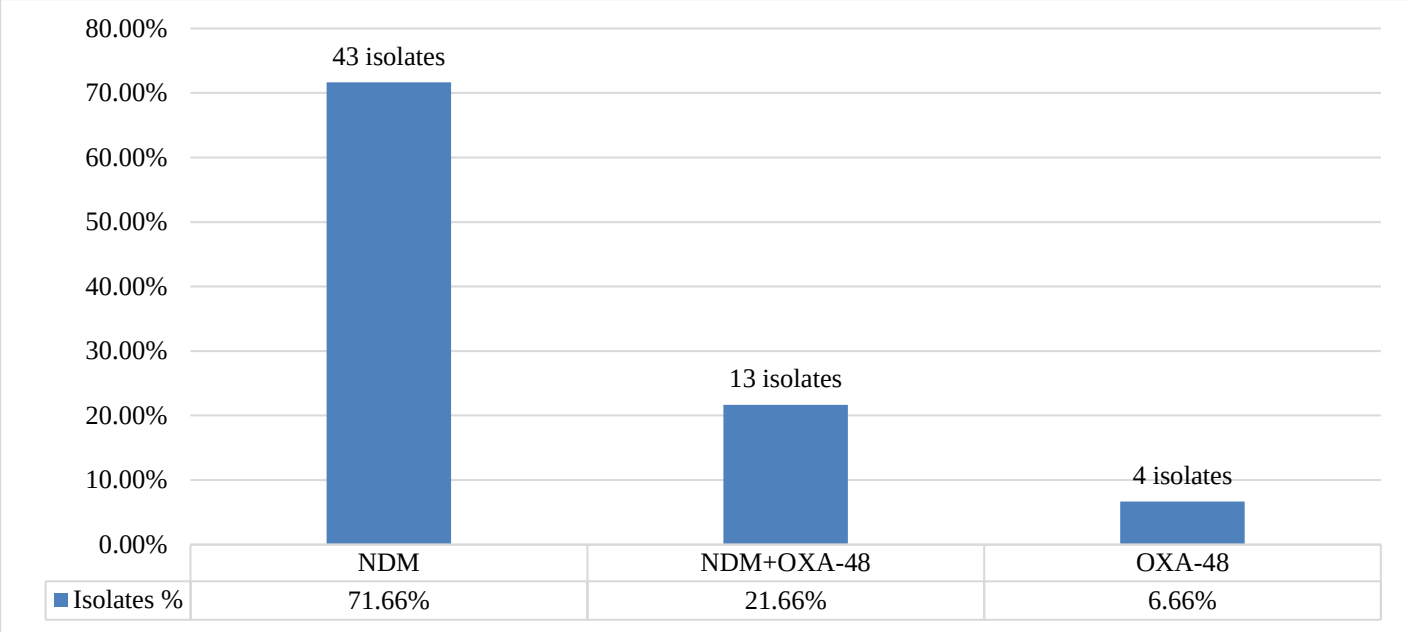


**Figure-I: Distribution (%) of carbapenem-resistant genes among CRE (n=60).**

## DISCUSSION

Carbapenem-resistant *Enterobacterales* (CRE) represent a major global health threat, primarily because of the limited treatment options available. The rise in CRE infections is concerning, especially given their prevalence in low-income countries where reliable data may be lacking, potentially underestimating the true extent of the problem. Rapid detection of carbapenem-resistance genes is crucial for guiding appropriate antibiotic therapy, thereby improving patient outcomes.[13] CRE is one of the top classes of drug-resistant bacteria, according to the World Health Organization (WHO), and it needs immediate attention and the development of novel antimicrobial agents.[14]

According to the current study, NDM was the most commonly detected gene among CRE isolates, found in 71.66% of isolates. Following this, both NDM and OXA-48 genes were detected in 21.66% of isolates, while the OXA-48 gene alone was found in 6.66% of isolates. Consequently, a significant portion of 56 CRE isolates (93.33%) were found to possess the NDM gene either alone or in combination with OXA-48. This high prevalence of NDM among CRE isolates is concerning. These results are consistent with a study conducted in Thailand between 2016 and 2018, which detected NDM in 65% of CRE isolates collected from urine, sputum and blood specimens.[15] Comparable findings were reported in a multicenter study conducted across 11 provinces in Thailand from 2012 to 2017, where 64% of CRE isolates were found to possess the NDM gene either alone or in combination with other carbapenemases.[16] In another study conducted in India,

NDM was the most prevalent gene detected in 63% of carbapenemase-producing *Enterobacterales*.[17]

The present study revealed that all CRE isolates containing the OXA-48 gene alone were susceptible to ceftazidime-avibactam. Conversely, all CRE isolates carrying the NDM gene demonstrated resistance to CZA.

It is already known that ceftazidime-avibactam demonstrates promising activity against numerous significant Gram-negative bacteria, including many strains of *Enterobacterales* producing extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, *Klebsiella pneumoniae* carbapenemases (KPCs), and OXA-48 enzymes, as well as multidrug-resistant *Pseudomonas aeruginosa* isolates. However, it is ineffective against strains that produce metallo-β-lactamases.[18] The absence of ceftazidime-avibactam effectiveness against MBL-positive isolates is attributed to the hydrolysis of both ceftazidime and avibactam by the MBL class of beta-lactamases.[19] The current study's results further validated this assertion.

The results also align with a study conducted in Turkey encompassing carbapenemase-producing *Enterobacterales* bloodstream isolates from May 2010 to September 2018 which concluded that all strains resistant to ceftazidime-avibactam were producers of NDM.[20]

In a separate study, isolates recovered from respiratory samples gathered across Africa/Middle East, Asia/South Pacific, Europe, and Latin America from 2016 to 2018, as part of the Antimicrobial Testing Leadership and Surveillance (ATLAS) program, it was reported that susceptibility to ceftazidime-avibactam among carbapenemase-positive/MBL-negative *Enterobacterales* isolates remained notably high at 98.9%.[21] Likewise, in another study conducted between 2012 and 2015 in the same regions, the susceptibility of ceftazidime-avibactam among CRE isolates that were OXA-48 positive and MBL-negative was reported to be 99.2%.[22] This conclusion was clearly illustrated in the current study. Therefore, our data indicate that ceftazidime-avibactam serves as a promising alternative therapeutic option for CRE isolates that are positive for OXA-48 but negative for NDM.

The current study found no discordance between ceftazidime-avibactam susceptibility assessed by disk diffusion testing and MIC testing. This indicates that disk diffusion testing can be considered a reliable method for evaluating in vitro susceptibility of ceftazidime-avibactam.

The present study also unveiled a notable prevalence of colistin resistance among *Klebsiella pneumoniae* CRE isolates, reaching 29.41%. In a previous study conducted in Turkey, which involved 150 carbapenem-resistant *K. pneumoniae* isolates recovered from clinical specimens between 2018 and 2021, it was found that a significant proportion, 52%, of isolates exhibited resistance to colistin.[23] In another multicenter study conducted in Italy, where 89 carbapenem-resistant *K. pneumoniae* isolates were collected, it was observed that 13% of them were resistant to colistin.[24]

The results of the current study also revealed a noteworthy susceptibility rate of fosfomycin among CRE isolates, reaching 73.33%, which is quite intriguing. Traditionally, fosfomycin has been mainly utilized in oral formulations for treating lower urinary tract infections. However, it is fortunate that fosfomycin has remained effective against certain CRE isolates. Various studies in the past have also demonstrated the effectiveness of fosfomycin against CREs.[25]

Considering the high prevalence of NDM-positive CRE isolates and the high susceptibility rate of fosfomycin among CRE isolates observed in this investigation, it can be interferred that fosfomycin might be a suitable choice as an empirical antibiotic option for patients developing bacteremia caused by CRE, pending confirmation through culture results. However, we recommend restricting the use of ceftazidime-avibactam to isolates harboring OXA-48 alone.

The research also supports the adoption of an antimicrobial resistance surveillance system. This system should employ rapid diagnostic tests to promptly identify the emergence of resistant genes and monitor antimicrobial usage. This proactive strategy aims to improve clinical outcomes for CRE infections, considering their severe nature and the limited treatment options presently accessible. It is also important to acknowledge that susceptibility rates of these antibiotics may vary between hospitals and their specific settings. Additionally, given the escalating resistance rates, continuous surveillance studies are essential, along with the generation of site-specific antibiograms.

There are several limitations to the current study. It is a single-centered study, which may limit the

generalizability of the findings. Further molecular analysis of colistin-resistant *Klebsiella pneumoniae* isolates was not conducted due to budget constraints. Furthermore, the clinical response of patients receiving ceftazidime-avibactam was not monitored as part of this study, which could provide valuable insights into its efficacy in clinical practice.

## CONCLUSION

The study concluded that NDM is the most prevalent resistant gene among CRE isolates. Ceftazidime-avibactam demonstrates good activity against CREs carrying the OXA-48 gene alone. However, it lacks efficacy against CRE isolates containing the NDM gene, either alone or in combination with the OXA-48 gene. The study also suggests that disk diffusion can be considered a reliable method for assessing the susceptibility of ceftazidime-avibactam. Additionally, the study highlights the importance of rapid carbapenemase gene detection in improving clinical outcomes by enabling timely selection of effective treatments, reducing the risk of treatment failure, and enhancing patient outcomes.

## CONFLICT OF INTEREST

None

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## AUTHOR CONTRIBUTION

**Aqib Sultan:** Main conception of the study, Acquisition, analysis and interpretation of data and Drafting the work, revising it critically for important intellectual content, final approval, agreement to be accountable for all aspects of the work

**Nida Safdar:** Acquisition, analysis and interpretation of data and Drafting the work, final approval, agreement to be accountable for all aspects of the work

**Nasrullah Malik:** Ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, final approval, agreement to be accountable for all aspects of the work

**Summiya Nizamuddin:** Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, Final approval of the version to be published

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