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Synergistic Activity of Ceftazidime-Avibactam-Aztreonam Combination Against Metallo-Betalactamase Producing Enterobacterales Isolates

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KEYWORDS

Carbapenem-resistant Enterobacterales (CRE), Metallo-β-lactamase (MBL), New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), and imipenemase (IMP), Ceftazidime-avibactam (CZA), aztreonam (ATM)

ABSTRACT:

Introduction: Carbapenem-resistant Enterobacterales (CRE) pose a major therapeutic challenge due to diverse resistance mechanisms, particularly metallo-β-lactamases (MBLs) such as NDM, VIM, and IMP. Ceftazidime-avibactam (CZA) is ineffective against MBLs, while aztreonam (ATM) retains activity but is vulnerable to ESBLs and AmpC enzymes. The CZA–ATM combination offers a rational strategy to overcome these limitations.

Aim: To evaluate the synergistic activity of ceftazidime-avibactam and aztreonam against MBL-producing CRE isolates.

Methods: A cross-sectional study was conducted over four months in the Department of Microbiology, MGM Medical College & Hospital, Chh Sambhajinagar. Ninety-six CRE isolates from clinical specimens were identified using Vitek 2 Compact. Carbapenemase production was confirmed by Trurapid OKNVI Resist 5 kit. Synergy testing of CZA–ATM was performed using the Broth Disc Elution method.

Results: Of 96 CRE isolates, Klebsiella pneumoniae (47.9%) and Escherichia coli (31.3%) predominated. Carbapenemase profiling revealed OXA-48-like enzymes (44.8%) as most common, followed by MBLs (32.3%) and KPC (19.8%). Among 31 MBL-producing isolates, synergy with CZA–ATM was observed in 74.2%, with K. pneumoniae showing the highest response (69.6%). The majority of isolates were recovered from pus (39.6%) and urine (25%) specimens, with Medicine (29.2%) and Surgery (22.9%) departments contributing most cases.

Conclusion: The CZA–ATM combination demonstrated significant synergistic activity against MBL-producing CRE, particularly K. pneumoniae, restoring susceptibility in over 70% of isolates. These findings support its potential as a salvage therapy in clinical practice, though persistent resistance in a subset of strains underscores the need for continued surveillance and cautious application.

Introduction

Carbapenem-resistant Enterobacterales (CRE) are defined as members of the Enterobacterales order that exhibit resistance to at least one carbapenem, such as ertapenem, meropenem, or imipenem[1]. Carbapenems have long been regarded as the most reliable last-resort agents for the treatment of severe bacterial infections

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because of their broad-spectrum activity against Gram-positive and Gram-negative organisms, including anaerobes. However, in recent years, carbapenem resistance among Gram-negative bacteria has been increasingly reported in healthcare-associated infections across India, posing a major therapeutic challenge[2]. The mechanisms of resistance are diverse, including the production of β-lactamases capable of hydrolyzing carbapenems, and the combined effect of cephalosporinase production with reduced permeability of the bacterial outer membrane[2].

Among these mechanisms, the emergence of class B metallo-β-lactamases (MBLs), such as New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), and imipenemase (IMP), has become a global concern[3]. These enzymes confer resistance to nearly all β-lactam antibiotics, including carbapenems, and are often plasmid-mediated, facilitating rapid dissemination. Furthermore, MBLs are frequently co-produced with extended-spectrum β-lactamases (ESBLs) and other resistance determinants, resulting in multidrug-resistant phenotypes that severely limit therapeutic options[3].

Ceftazidime-avibactam (CZA) is a novel combination of the third-generation cephalosporin ceftazidime and the non-β-lactam β-lactamase inhibitor avibactam. This agent is highly effective against ESBLs, AmpC β-lactamases, and class A carbapenemases such as Klebsiella pneumoniae carbapenemase (KPC), as well as some class D enzymes like OXA-48-like variants. However, avibactam does not inhibit MBLs, leaving CZA ineffective against NDM, VIM, and IMP producers[2]. Aztreonam (ATM), on the other hand, is intrinsically stable against hydrolysis by MBLs but is vulnerable to ESBLs and AmpC enzymes, which are often co-expressed in MBL-producing strains. The combination of aztreonam with ceftazidime-avibactam therefore provides a rational therapeutic strategy: avibactam protects aztreonam from ESBLs and AmpC enzymes, while aztreonam retains activity against MBLs[2].

This synergistic effect has been demonstrated in several recent studies, where the ceftazidime-avibactam–aztreonam combination restored susceptibility in otherwise extensively drug-resistant Enterobacterales isolates[4,5]. Given the increasing prevalence of MBL-producing CRE in India and worldwide, and the paucity of effective treatment options, evaluating this combination in local clinical isolates is of significant clinical importance. Additionally, accurate detection and characterization of resistance mechanisms guides therapeutic strategies, underscoring the relevance of evaluating novel combinations in clinical practice[6]. On this background, the present study was designed to assess the prevalence of MBL-producing CRE and to evaluate the synergistic activity of ceftazidime-avibactam with aztreonam against these isolates.

Aim & objectives

Aim

To evaluate the synergistic activity of the ceftazidime-avibactam and aztreonam combination against metallo-β-lactamase (MBL) producing Enterobacterales isolates

Objectives

1. To estimate the prevalence of metallo-β-lactamase (MBL) producing CREs.
2. To perform synergy testing of ceftazidime-avibactam in combination with aztreonam against MBL-producing carbapenem-resistant Enterobacterales (CRE).

Material and Methods

This was a cross-sectional observational study conducted in the Department of Microbiology at Mahatma Gandhi Mission's Medical College and Hospital, Chh Sambhajinagar. The study was carried out over four months following approval from the Institutional Ethics Committee. A total of 96 clinical samples fulfilling the inclusion and exclusion criteria were enrolled.

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Inclusion criteria:

All carbapenem-resistant Enterobacterales (CRE) isolates recovered from various clinical specimens received during the study period.

Exclusion criteria:

Repeat isolates from the same patient and specimen during the same period.

Isolation and Identification

Clinical samples were inoculated onto Blood Agar and MacConkey Agar and incubated at 37°C for 18–24 hours. Colonies were examined for morphology, and isolates were identified using the Vitek 2 Compact system (bioMérieux). Antimicrobial susceptibility testing (AST) was performed according to CLSI guidelines[7].

Screening and Confirmation of CRE

Isolates resistant to one or more carbapenems (ertapenem, imipenem, or meropenem) were labeled as CRE[1]. Confirmatory testing for carbapenemase production was performed using the Trurapid OKNVI Resist 5 kit (3B BlackBio Biotech), a rapid lateral immunochromatographic assay capable of detecting OXA-48, KPC, NDM, VIM, and IMP enzymes[3].

Synergy Testing

All isolates confirmed as MBL-producing CREs (NDM, VIM, IMP) were subjected to synergy testing. The synergistic activity of ceftazidime-avibactam CZA) in combination with aztreonam (ATM) was evaluated using the Broth Disc Elution (BDE) method[7], which has been validated as a reliable and reproducible approach for detecting ATM–CZA synergy in clinical laboratories.

In this method, Cation adjusted Mueller-Hinton broth(CAMHB) tubes were prepared with discs containing ceftazidime-avibactam(30/20µg) and aztreonam(30µg). The test organism was inoculated to achieve a standardized inoculum density (0.5 McFarland). Tubes containing CZA alone, ATM alone, the combination (CZA+ATM) and one growth control tube with no antimicrobial disc were incubated at 37°C for 18–24 hours. Growth inhibition was assessed visually and compared across the three conditions. Synergy was defined as restoration of susceptibility to aztreonam in the presence of ceftazidime-avibactam, indicated by a clear reduction in growth compared to either agent alone.

This method was chosen because it is simple, cost-effective, and demonstrates high sensitivity and specificity for detecting synergy in NDM-producing Enterobacterales, outperforming other disc diffusion-based approaches.

Quality Control

Reference strains were included for quality control: *Klebsiella pneumoniae* ATCC BAA-1705, *Klebsiella pneumoniae* ATCC BAA-1706, and *Escherichia coli* ATCC 25922. [7]

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using **SPSS version 27.0**. Mean and standard deviation were calculated for quantitative variables, while proportions were determined for categorical variables. Results were represented in tabular form and supplemented with graphical visualizations such as bar diagrams

Observation & Result

Table 1: Department-wise Distribution

Sr No	Department	Number of cases n	Percentage %
1	Surgery	22	22.90%
2	Medicine	28	29.20%
3	Nephrology	10	10.40%
4	Urology	12	12.50%
5	Pulmonary medicine	9	9.40%
6	OBG	8	8.30%

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7	**Ortho**	7	7.30%
Total		96	**100%**

Departmental analysis showed that most CRE cases originated from the Medicine ward (29.2%) and Surgery ward (22.9%). Urology (12.5%) and Nephrology (10.4%) also contributed significantly, while Pulmonary Medicine (9.4%), Obstetrics and Gynecology (8.3%), and Orthopedics (7.3%) accounted for smaller proportions. These findings suggest that high patient loads and invasive procedures in Medicine and Surgery departments may predispose to CRE infections.

Graph 1: Department-wise Distribution

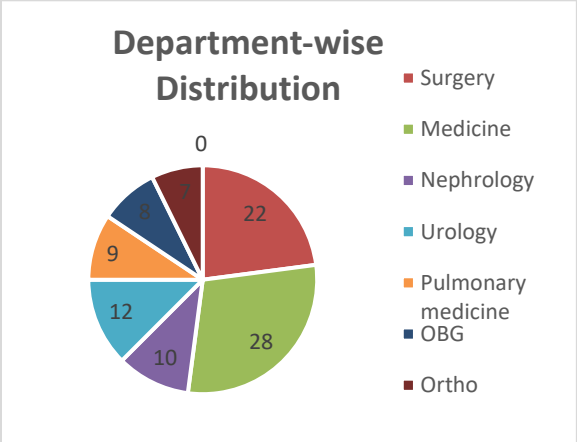

Table 2: Sample Type Distribution

Sr No	Sample Type	Number of cases n	Percentage %
1	**Pus**	38	39.60%
2	**Urine**	24	25.00%
3	**Blood**	14	14.60%
4	**Endotracheal (ET)**	12	12.50%
5	**Sputum**	8	8.30%
Total		96	**100%**

The majority of CRE isolates were recovered from pus samples (39.6%), followed by urine (25%) and blood (14.6%). Endotracheal aspirates contributed 12.5%, while sputum samples accounted for 8.3%. This distribution reflects the predominance of soft tissue and urinary tract infections as sources of CRE in the study population.

Table 3: Distribution of Enterobacterales Isolates

Sr No	Species	Number of isolates (n)	Percentage (%)
1	Klebsiella pneumoniae	46	47.9 %
2	Escherichia coli	30	31.3 %
3	Enterobacter cloacae complex	12	12.6 %
4	Citrobacter freundii	2	2 %
5	Others (Proteus, Serratia)	6	6.2 %
Total N (%)		**96**	**100 %**

Among the 96 carbapenem-resistant Enterobacterales (CRE) isolates studied, *Klebsiella pneumoniae* was the most frequently encountered species, accounting for 47.9% of cases. *Escherichia coli* followed at 31.3%, while *Enterobacter cloacae complex* contributed 12.6%. Less common isolates included Citrobacter freundii (2%) and other genera such as *Proteus* and *Serratia* (6.2%). This distribution highlights the predominance of *K. pneumoniae* and *E. coli* in CRE infections.

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Table 4: MBL producers amongst CRE

Sr No	CREs	Number of isolates (n)	Percentage (%)
1	Metallo-β-lactamase (NDM, VIM, IMP)	31	32.3 %
	OXA-48-like	43	44.8 %
2	KPC	19	19.8 %
3	Others	3	3.1 %
Total N (%)		**96**	**100 %**

Carbapenemase profiling revealed that OXA-48-like enzymes were the most prevalent, detected in 44.8% of isolates. Metallo-β-lactamases (NDM, VIM, IMP) were present in 32.3% of cases, while Klebsiella pneumoniae carbapenemase (KPC) accounted for 19.8%. Other mechanisms were rare, comprising only 3.1% of isolates. This distribution underscores the significant burden of OXA-48-like carbapenemases, with MBLs forming a substantial proportion that poses major therapeutic challenges.

Table 5: Synergy with Ceftazidime-Avibactam + Aztreonam amongst MBL-producers

Sr No	Result of synergy test	Number of isolates (n)	Percentage (%)
1	Synergy observed	23	74.20%
2	No synergy observed	8	25.80%
Total N (%)		**31**	**100%**

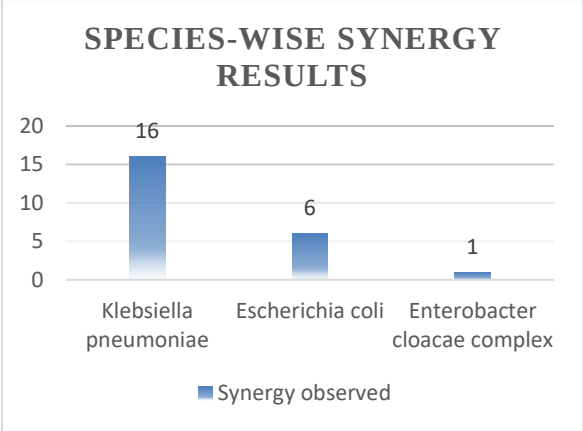
Of the 31 MBL-producing isolates tested, synergy between ceftazidime-avibactam and aztreonam was observed in 74.2% (23 isolates). The remaining 25.8% (8 isolates) did not exhibit synergy. These findings suggest that the CZA–ATM combination restored susceptibility in the majority of MBL-producing CREs, though a notable minority remained resistant.

Table 6: Species-wise Synergy Results

Sr No	Species	Number of cases n	Percentage %
1	*Klebsiella pneumoniae*	16	69.6 %
2	*Escherichia coli*	6	26.1 %
3	*Enterobacter cloacae complex*	1	4.3 %
Total		**23**	**100 %**

Species-specific analysis of synergy revealed that *Klebsiella pneumoniae* accounted for the majority of synergistic responses, with 16 isolates (69.6%). *Escherichia coli* showed synergy in 6 isolates (26.1%), while *Enterobacter cloacae complex* demonstrated synergy in only 1 isolate (4.3%). This indicates that the CZA–ATM combination was most effective against *K. pneumoniae*, the predominant CRE species.

Graph 2: Species-wise Synergy Results



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Discussion

In the present study, *Klebsiella pneumoniae* was the predominant carbapenem-resistant Enterobacterales (CRE) isolate (47.9%), followed by *Escherichia coli* (31.3%). This distribution is consistent with national surveillance data from India, where *K. pneumoniae* has been repeatedly identified as the leading CRE pathogen in hospital settings[1]. Similar findings were reported by Rajan et al., who observed *K. pneumoniae* as the most frequent CRE isolate in their multicentric study[2]. The predominance of E. coli as the second most common species also aligns with global epidemiology, reflecting its role in urinary tract and bloodstream infections[3].

In terms of sample distribution, pus (39.6%) and urine (25%) were the most common sources of CRE isolates. This finding is in line with Koneman's diagnostic microbiology text, which emphasizes soft tissue and urinary tract infections as frequent sites of multidrug-resistant Gram-negative infections[6,7]. Bloodstream infections accounted for 14.6%, comparable to rates reported in multicentric Indian studies, underscoring the clinical severity of CRE bacteremia[1].

Departmental analysis showed that Medicine (29.2%) and Surgery (22.9%) wards contributed the highest number of CRE cases. This is consistent with previous hospital-based studies, where high patient turnover and invasive procedures in these departments were associated with increased CRE isolation[2]. The contribution from Urology and Nephrology also reflects the burden of urinary tract and dialysis-related infections, which are known risk factors for multidrug-resistant Gram-negative infections[3].

Carbapenemase profiling revealed that OXA-48-like enzymes were most prevalent (44.8%), followed by metallo-β-lactamases (NDM, VIM, IMP) at 32.3%. This is in agreement with ICMR surveillance reports, which highlight OXA-48-like variants as the dominant carbapenemase in Indian hospitals[1]. However, the significant proportion of MBLs in our study mirrors findings from Khan et al., who reported high rates of NDM among CRE isolates in North India[5]. The coexistence of multiple carbapenemase types underscores the complexity of resistance mechanisms and the need for tailored therapeutic strategies.

Synergy testing demonstrated that ceftazidime-avibactam (CZA) combined with aztreonam (ATM) restored susceptibility in 74.2% of MBL-producing isolates. This finding is comparable to the study by Rawson et al., who reported successful restoration of aztreonam activity in the majority of NDM-producing strains using the CZA–ATM combination[3]. Similarly, Khan S et al. documented synergy in approximately 70–80% of MBL-producing Enterobacterales, supporting the reproducibility of this approach across different settings[5]. The 25.8% of isolates that did not exhibit synergy in our study highlight the variability in response, possibly due to co-expression of additional resistance determinants, as noted in prior reports[2].

Species-wise analysis revealed that synergy was most pronounced in *K. pneumoniae* (69.6%), followed by *E. coli* (26.1%) and *Enterobacter cloacae complex* (4.3%). This pattern is consistent with Rajan et al., who observed higher synergy rates in K. pneumoniae compared to other Enterobacterales[2]. The lower synergy in *E. coli* and *Enterobacter* may reflect differences in resistance gene carriage and plasmid-mediated co-resistance, as suggested by Khan A et al[4].

Overall, the findings of this study corroborate existing literature on the epidemiology of CRE in India, while reinforcing the therapeutic potential of the CZA–ATM combination against MBL-producing isolates. The high synergy rates observed, particularly in K. pneumoniae, support its consideration as a salvage therapy in clinical practice. However, the persistence of resistance in a subset of isolates emphasizes the need for ongoing surveillance, molecular characterization, and cautious clinical application.

Conclusion

This study demonstrates that *Klebsiella pneumoniae* is the predominant carbapenem-resistant Enterobacterales isolate, with OXA-48-like carbapenemases most common and the prevalence of

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metallo-β-lactamases is 32.3% forming a significant proportion. The ceftazidime-avibactam–aztreonam combination restored susceptibility in over 70% of MBL-producing isolates, particularly against *K. pneumoniae*. These findings support the clinical potential of this combination as a salvage therapy, while highlighting the need for continued surveillance and cautious application due to persistent resistance in a subset of strains.

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