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Original Article

Microbiology Section

Evaluation of In-vitro Synergy of Ceftazidime-Avibactam and Aztreonam against Carbapenem-resistant Enterobacterales Isolates from ICU Patients in a Tertiary Care Hospital: A Cross-sectional Study

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ABSTRACT

Introduction: Carbapenemase-Resistant Enterobacterales (CRE) show rapid global dissemination and pose a significant therapeutic challenge due to limited effective treatment options. Although the combination of Ceftazidime–Avibactam (CZA) and Aztreonam (ATM) has emerged as a promising strategy against such isolates, standardised and easily implementable laboratory methods for detecting in-vitro synergy remain limited.

Aim: To evaluate in-vitro synergy of CZA and ATM against CRE isolates from various clinical samples.

Materials and methods: This cross-sectional study was conducted in the Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India, from June 2024 to December 2024. A total of 121 non duplicate, monomicrobial Gram-negative isolates obtained from Intensive Care Unit (ICU) patients, all of which were resistant to carbapenems (imipenem, meropenem), were included in the study. Identification was performed using Gram staining, biochemical tests, and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Carbapenemase production was assessed by the modified Carbapenem Inactivation Method (mCIM) and Ethylene-diamine-tetraacetic Acid (EDTA)-modified Carbapenem Inactivation Method (eCIM). Synergy between CZA an ATM was tested by the Double Disc Diffusion Method (DDDM) and CZA+ATM, CZA + ATM Broth Disc Elution Method (BDEM). Demographic and clinical data, such as age, sex, and co-morbidities, risk factors, were recorded.

Results: Out of 121 isolates of CRE, 76 (62.8%) isolates were identified as *K. pneumoniae* followed by 32 (26.5%) *E. coli* and 13 (10.7%) *E. cloacae*. Also, mCIM in combination with eCIM tested positive in 92 (76%) isolates, whereas mCIM alone tested positive in 29 (24%) isolates. Synergy was observed in 89 isolates by the DDDM, whereas CZA + ATM BDEM showed synergy in 92 isolates.

Conclusion: This study demonstrated a high prevalence of CRE, predominantly in *Klebsiella pneumoniae*, among ICU patients, with carbapenemase production confirmed in the majority of isolates by mCIM/eCIM. A high rate of in-vitro synergy was observed between CZA and ATM using both DDDM and BDEMs, with excellent concordance between the two techniques. These findings support the routine use of phenotypic synergy testing in clinical laboratories to guide effective therapeutic options against CRE.

Keywords: Antibacterial agents, Drug resistance, Drug synergism, Intensive care units

INTRODUCTION

The CRE pose a significant public health concern due to their Multidrug-Resistant (MDR) nature. These bacteria cause healthcare-associated infections, particularly in ICU patients. Infections caused by CRE are usually associated with increased morbidity and mortality, prolonged hospital or ICU stays, and elevated healthcare costs, thereby imposing a significant burden on both patients and healthcare systems [1,2]. CRE strains exhibit resistance to nearly all available antibiotics, making treatment options extremely limited. *E. coli* and *Klebsiella* spp. are considered to be the most important Enterobacterales that commonly cause health-associated and community-associated infections [3]. Hence, early detection of CRE is crucial for targeted antimicrobial therapy and for timely and effective infection control.

The resistance is predominantly caused by the production of carbapenemase enzymes. Metallo-beta-Lactamases (MBL) are carbapenemases that hydrolyse almost all β-lactam antibiotics, except monobactams, such as ATM. They are not inhibited by β-lactamase inhibitors, such as clavulanic acid, tazobactam, or sulbactam [4]. ATM remains stable against MBL-producing Enterobacterales; there is often coproduction of other enzymes, such as serine carbapenemases, Extended-Spectrum beta-Lactamases (ESBL) and AmpC beta-lactamases, which can result in ATM resistance. The combination of CZA and ATM is an engaging alternative because avibactam can deactivate other β-lactamases, thereby activating ATM. CRE often produce MBLs, which hydrolyse almost all beta-lactams but not monobactams such as ATM. However, most MBL-producing isolates also co-produce other β-lactamases (ESBLs, AmpC, or serine carbapenemases), which inactivate ATM, rendering it ineffective. CZA is active against class A, class C, and some class D β-lactamases but not against MBLs. Therefore, when used in combination, avibactam inhibits the co-produced ESBLs and AmpC enzymes, thereby protecting ATM, which remains active against MBL producers. This complementary mechanism makes the CZA+ATM combination a rational therapeutic option against MBL-producing CRE isolates [5,6].

The novelty of this study lies in evaluating the in-vitro synergy of CZA and ATM against CRE isolates specifically from ICU patients in a tertiary care hospital, using both the DDDM and BDEM. While previous studies have reported synergy using limited methods or non ICU isolates [7-9], this study highlights the concordance and

potential utility of these two practical laboratory approaches for routine diagnostic use in a high-burden clinical setting.

The study aimed to evaluate in-vitro synergy of CZA and ATM against CRE isolates from various clinical samples. The primary objectives of the study were to evaluate the in-vitro synergy of CZA and ATM against CRE isolates using the DDDM and CZA+ATM BDEM, and the secondary objectives were to compare the concordance between DDDM and BDEM for synergy detection and to determine the distribution of carbapenemase types (mCIM/eCIM) among CRE isolates.

MATERIALS AND METHODS

This cross-sectional study was carried out in Sanjay Gandhi Postgraduate Institute of Medical Sciences in Lucknow, Uttar Pradesh, India, from June 2024 to December 2024. The study was conducted after obtaining the Institutional Ethical Committee, SGPGIMS (IEC, SGPGIMS) approval with Letter number- 2021-48-EMP-EXP Dated November 29, 2021.

Inclusion criteria: All consecutive, non duplicate, monomicrobial Gram-negative isolates obtained from blood, sterile body fluids, catheter tips, pus, sputum, and urine samples of ICU patients, with specimens collected from patients of both genders and all age groups, were included. Only isolates resistant to carbapenems (imipenem and meropenem), as determined by disc diffusion and the VITEK® 2 system, were included in the study.

Exclusion criteria: Duplicate isolates from the same patient were excluded from the study.

Study Procedure

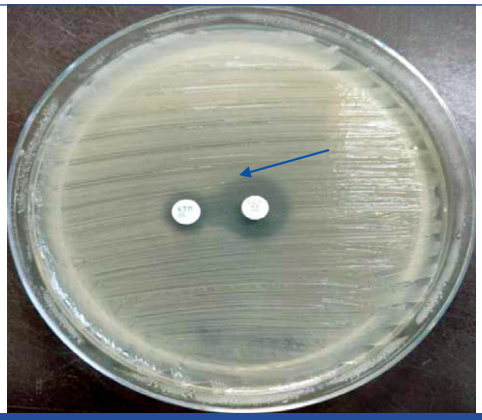
Sample collection and bacterial culture: The specimens were collected from patients of both genders and all age groups. Clinical and demographic data for each patient were obtained from the hospital information system. Gram staining was performed on all samples except the urine specimen. Then, specimens of pus, sterile body fluids and sputum were inoculated onto Blood, MacConkey agar plates, whereas, for urine specimens, the first wet mount was prepared then subsequently cultured on HiChrome agar, all following standard microbiological protocols. The plates were incubated aerobically at 37°C for 24 hours. Blood samples and sterile body fluids were processed using BACTEC FX blood culture bottles (BD, USA) and incubated at 35°C for up to five days. Once the system flagged a positive culture, a Gram stain was performed, and the broth was subcultured onto Blood and MacConkey agar, which were then incubated aerobically at 37°C for 24 hours. Isolates were identified using Gram staining, biochemical reactions (indole, methyl red, Voges-Proskauer, citrate utilisation, urease, motility, oxidase and triple sugar iron agar reactions). Oxidase testing was also performed to differentiate non fermenters, and automated identification was carried out using MALDI-TOF MS with the Biotyper system according to the manufacturer's recommendations (bioMérieux, USA). Only Enterobacterales isolates underwent further testing to detect MBL production and to assess antibiotic synergy.

Antibiotic Susceptibility Tests (AST): Routine antibiotic susceptibility tests were performed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (HiMedia, Mumbai, India) and evaluated by recommendations of the Clinical Laboratory Standards Institute (CLSI) guideline, using commercially available antibiotic discs procured from Hi-Media (Mumbai, India). Isolates that exhibited resistance to imipenem and meropenem (Zone of inhibition of ≤19 mm), or demonstrated high Minimum Inhibitory Concentrations (MICs) in VITEK 2, were chosen for further analysis [10].

Phenotypic tests for carbapenemases: The mCIM and eCIM were employed to detect and differentiate carbapenemase enzymes produced by bacteria. The testing was performed in accordance with the CLSI 2024 guidelines for antimicrobial susceptibility testing [10].

Test for Ceftazidime-Avibactum (CZA), and Aztreonam (ATM) synergy

Double Disc Diffusion Method (DDDM): The MHA plate was swabbed using a bacterial suspension adjusted to a 0.5 McFarland standard. A 30 µg ATM disc (HiMedia Laboratories, India) and a 30 µg/20 µg ceftazidime–avibactam (CAZ-AVI) disc (HiMedia Laboratories, India) were placed 15 mm apart (centre to centre) on the agar. The plate was incubated overnight at 37 °C for 16-18 hours [11]. For quality control, *Escherichia coli* ATCC 25922 and *Klebsiella pneumonie* BAA-1705 were used as negative and positive control strains, respectively. Synergy was interpreted by an enlargement of the inhibition zone around the ATM disc extending toward the CAZ-AVI disc, or by the presence of a characteristic "keyhole"-shaped zone of inhibition between the two discs [Table/Fig-1].



[Table/Fig-1]: Double Disc Diffusion Method (DDDM) showing synergy between Ceftazidime–Avibactam (CZA) and Aztreonam (ATM).
*Blue arrow indicates enlargement of the aztreonam inhibition zone towards the CZA disc, indicating synergy ("keyhole" effect)

Ceftazidime-avibactum (CZA) + Aztreonam (ATM) Broth Disc Elution Method (BDEM): A 30-µg ATM disk (HiMedia Laboratories, India), a 30/20-µg CZA disk (HiMedia Laboratories, India), both disks in combination, and no disks {Growth Control (GC)} were added to 4 separate 5-mL Cation-Adjusted Mueller-Hinton Broth (CA-MHB) tubes. The final concentrations in the tubes were 6 µg/mL ATM and 6/4 µg/mL CZA, individually or in combination. These concentrations were chosen in accordance with CLSI guidelines (CLSI, 34th edition, 2024) [10]. Then, the tubes were incubated for 30 to 60 minutes at room temperature to allow diffusion of antimicrobials from disk(s). A 0.5 McFarland standard was prepared from an overnight subculture on blood agar plates of the bacterial isolate in 5-mL saline tubes, and a 10-µL inoculating loop was used to streak the cultures onto purity plates. A 25 µL of McFarland suspension was added to each broth tube (final bacterial concentration in each tube, 7.5×10^5 CFU/mL). The tubes were then vigorously vortexed to evenly mix the bacteria and incubated at 35±2°C in ambient air for 16-20 hours. After overnight incubation, tubes were assessed for no growth (susceptible) or growth (not susceptible) at 6/6/4 µg/mL of ATM-CZA [12]. The quality control strains were *E. coli* ATCC 25922, *K. pneumoniae* ATCC BAA-1705, and *K. pneumoniae* ATCC BAA-2146.

STATISTICAL ANALYSIS

Data were entered into Microsoft Excel and analysed using IBM Statistical Package for the Social Sciences (SPSS) Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarise the data. Categorical variables were expressed as frequencies and percentages.

RESULTS

A total of 121 CRE isolates were included in the study. The majority of isolates were obtained from male patients 68 (56.2%), with a median patient age of 45 years. Haematological malignancies were the most common underlying co-morbidity, at 29.8% (36/121), followed by solid organ malignancies 23.1% (28/121). Prior antibiotic

exposure was seen in 54 (44.6%) and recent hospitalisation in 34 (28.1%) were the most frequently identified risk factors among the study population [Table/Fig-2].

Variables	n (%)
Gender	
Males	68 (56.2)
Females	53 (43.8)
Age (years)	
20-40	43 (35.5)
41-60	57 (47.1)
≥61	21 (17.4)
Underlying co-morbidities	
Diabetes mellitus	25 (20.7)
Chronic kidney disease	12 (9.9)
Chronic liver disease	11 (9.1)
Chronic lung disease	9 (7.4)
Haematological malignancy/Immunosuppressive therapy	36 (29.8)
Solid organ malignancy/Immunosuppressive therapy	28 (23.1)
Risk factors	
Central venous catheter	6 (5)
Mechanical ventilation	8 (6.6)
Indwelling Foley's catheter	19 (15.7)
Prior antibiotic use (Carbapenem/Cephalosporin)	54 (44.6)
Prior hospitalisation (within last 30 days)	34 (28.1)

[Table/Fig-2]: Demographic and clinical characteristics of the study cohort.

Most CRE isolates were recovered from blood cultures, 41.3% (50/121), followed by urine and wound swab samples 18.1% each (22/121). *Klebsiella pneumoniae* was the predominant organism found in 76 (62.8%), followed by *Escherichia coli* in 32 (26.5%) and *Enterobacter cloacae* in 13 (10.7%) [Table/Fig-3].

Specimen (n=121)	*K. pneumoniae* (76)	*E. coli* (32)	*E. cloacae* (13)
Blood culture (50)	42 (55.26)	6 (18.75)	2 (15.38)
Urine (22)	7 (9.21)	11 (34.37)	4 (30.76)
Wound swab (22)	13 (17.10)	4 (12.5)	5 (38.46)
Fluid aspirate (15)	5 (6.57)	9 (28.12)	1 (7.69)
Sputum (6)	6 (7.89)	0	0
BAL (2)	2 (2.63)	0	0
CVC tip (1)	1 (1.31)	0	0
Pus aspirate (3)	0	2 (6.25)	1 (7.69)

[Table/Fig-3]: Distribution of CRE among clinical specimens.
BAL: Bronchoalveolar lavage fluid; CVC: Central venous catheter

All CRE isolates demonstrated complete resistance to cephalosporins and carbapenems. High resistance to amikacin was observed in *K. pneumoniae* (71%, 54/76) and *E. cloacae* (100%, 13/13). Colistin remained highly active against *K. pneumoniae* 95% (72/76), *E. coli* 90.6% (29/32), and *E. cloacae* (100%) isolates.

Among urinary isolates of *E. coli*, fosfomycin showed better activity (59.37%, 19/32) [Table/Fig-4].

Combined mCIM- eCIM was positive in 92 (76%) isolates, while mCIM alone was positive in 29 (24%) isolates. Using the DDDM, synergy was detected in all 32 (100%) *E. coli* isolates, 49 (64.5%) *K. pneumoniae* isolates, and 8 (61.6%) *E. cloacae* isolates [Table/Fig-5].

Overall, BDEM detected synergy in slightly more isolates (92) than DDDM (89). Using BDEM, synergy was observed in all 32 (100%) *E. coli* isolates and in the majority of 51 (67%) *K. pneumoniae* and 9 (69%) *E. cloacae* isolates [Table/Fig-6].

The overall concordance between the two methods was 96.74% [Table/Fig-7].

DISCUSSION

Carbapenems represent one of the last therapeutic options for managing infections caused by MDR Enterobacterales, since they generally withstand hydrolysis by ESBLs and AmpC beta-lactamases. In recent years, however, resistance to carbapenems among essential Enterobacterales has been rising, making CRE a major global health concern. Therefore, prompt identification of CRE guides appropriate antimicrobial therapy and to implementation of timely infection control interventions [13].

In this study, CRE infections were observed more frequently in males, with individuals aged 41-60 years being the most affected, followed by younger adults between 20 and 40 years. Among co-morbidities, haematological and solid organ malignancies were most commonly associated with CRE infection, consistent with the findings of Aiesh BM et al., who reported a higher prevalence of CRE among cancer patients [14]. Prior exposure to broad-spectrum antibiotics particularly carbapenems and cephalosporins, together with prolonged hospitalisation, emerged as significant risk factors. These results align with the systematic review by Palacios-Baena ZR et al., and highlight the critical role of antimicrobial stewardship in limiting the emergence and spread of CRE [15].

The majority of CRE isolates in this study were recovered from blood samples, whereas other Indian studies have reported urine as the predominant source [16-18]. This difference could be attributed to the patient population studied, as tertiary care centres often receive critically-ill and referred patients with systemic infections. Regarding the species distribution, *K. pneumoniae* accounted for the largest proportion of isolates, followed by *E. coli* and *E. cloacae*. These

	K. pneumoniae (76)		*E. coli* (32)		*E. cloacae* (13)	
Antibiotic	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Amikacin	22 (29)	54 (71)	19 (59)	13 (40)	0	13 (100)
Ceftazidime	0	76 (100)	0	32 (100)	0	13 (100)
Ceftriaxone	0	76 (100)	0	32 (100)	0	13 (100)
Ciprofloxacin	0	76 (100)	0	32 (100)	0	13 (100)
Cefoperazone+s albactum	0	76 (100)	0	32 (100)	0	13 (100)
Imipenem	0	76 (100)	0	32 (100)	0	13 (100)
Meropenem	0	76 (100)	0	32 (100)	0	13 (100)
Colistin	72 (95)	4 (5)	29 (90.6)	3 (9.3)	13 (100)	0
Minocycline	74 (97)	2 (2.6)	31 (96.9)	1 (3.1)	13 (100)	0
Nitrofurantoin	0	7 (9.2)	2 (6.25)	9 (28)	0	4 (30.8)
Fosfomycin	NT	NT	19 (59.37)	13 (40.62)	NT	NT

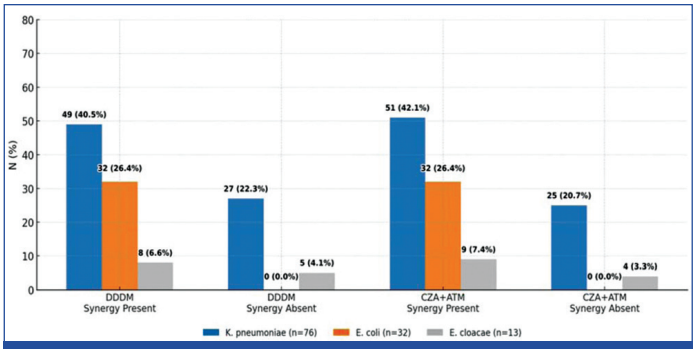
[Table/Fig-4]: Antimicrobial susceptibility profile of CRE isolates.
Nitrofurantoin- tested on isolates from urine samples only, Fosfomycin- tested on isolates of *E. coli* from urine samples only, tested by disc diffusion with oral formulation, CRE: Carbapenem-Resistant Enterobacterales; NT: Not tested

A. Carbapenemase detection by mCIM–eCIM	
Test Method	**n (%)**
mCIM Alone Positive	29 (24)
Combined mCIM–eCIM Positive	92 (76)

B. Double Disc Diffusion Method (DDDM)

Microorganism (n=121)	Ceftazidime/Avibactum		Aztreonam		Ceftazidime/Avibactum+Aztreonam Synergy
	Zone dia. (mm)	Susceptibility (≥21S,≤ 20R)	Zone dia. (mm)	Susceptibility (≥21S,18- 20I, ≤17R)	
K.pneumonie (n=76)					
19 isolates	15	R	10	R	Present (19)
17 isolates	17	R	6	R	Present (17)
13 isolates	13	R	6	R	Present (13)
15 isolates	6	R	6	R	Absent (15)
12 isolates	8	R	8	R	Absent (12)
Summary (n=76)	-	-	-	-	Present (49) 64.5%
					Absent (27) 35.5%
E.coli (n=32)					
14 isolates	14	R	11	R	Present (14)
10 isolates	11	R	7	R	Present (10)
8 isolates	9	R	5	R	Present (8)
Summary (n=32)	-	-	-	-	Present (32) 100%
					Absent (0) 0%
E.cloacae (n=13)					
8 isolates	20	R	17	R	Present (8)
5 isolates	14	R	8	R	Absent (5)
Summary (n=13)	-	-	-	-	Present (8) 61.5%
					Absent (5) 38.5%
Total (n=121)	-	-	-	-	Present (89) 73.6%
					Absent (32) 26.4%

[Table/Fig-5]: Carbapenemase detection by mCIM–eCIM and antimicrobial synergy testing by Double Disc Diffusion Method (DDDM) in CRE isolates.



[Table/Fig-6]: Interpretation of Double Disc Diffusion Method (DDDM) and Ceftazidime-avibactum (CZA) + Aztreonam (ATM) Broth Disc Elution Method (CZA+ATM).

Microorganism (n=121)	Double Disc Diffusion Method (DDDM)	Concordance rate%
K. pneumoniae (n=76)	49/51	96.07%
E. coli (n=32)	32/32	100%
E. cloacae (n=13)	8/9	88.88%

[Table/Fig-7]: Overall concordance rate of the Double Disc Diffusion Method (DDDM) with Aztreonam (ATM) + Ceftazidime-Avibactum (CZA) Broth Disc Elution Method (BDEM).

findings were consistent with earlier studies indicating a higher prevalence of carbapenem resistance in *Klebsiella* spp. compared to *E. coli* [19-21].

In this study, resistance to conventional antibiotics such as cephalosporins and carbapenems was widespread. Similar patterns have been documented in India, where MBL-producing *K. pneumoniae* showed complete resistance to third-generation cephalosporins, imipenem, ertapenem, and amoxicillin/clavulanate [22]. Colistin demonstrated the highest activity with susceptibility rates above 90%, while ATM showed universal resistance, a pattern consistent with observations reported from tertiary care centres across India [23] but differs from a Spanish study that observed approximately 18% susceptibility among CRE isolates [24]. These differences likely reflect regional variation in resistance mechanisms, particularly the prevalence of specific carbapenemase genes, and emphasise the value of local surveillance to guide both empirical and targeted therapy.

Phenotypic assays (mCIM/eCIM) confirmed carbapenemase production in 76% of isolates, highlighting their value as simple, cost-effective tools that can be applied even in resource-limited settings. Synergy between CZA and ATM was observed in 73.6% of isolates with the DDDM and in 76% with the BDEM. These results are consistent with those of Taha R et al., and Jayol A et al., who also reported high levels of synergy with the CZA+ATM combination [7,8]. By contrast, Sahu C et al., noted variability depending on the testing method, underscoring the importance of using standardised approaches to ensure reliable detection of synergy [9].

In the present study, 24% of isolates did not demonstrate synergy with the CZA+ATM combination, which may be explained by additional resistance mechanisms such as porin loss, efflux pump activity, or β-lactamase mutations. Similar findings were reported in a multicentre evaluation by Harris H et al., who showed that although broth disc elution testing reliably detected synergy, occasional discrepancies highlighted the complexity of underlying resistance mechanisms [12].

When comparing detection methods, the findings revealed excellent concordance (>95%) between the DDDM and the BDEM. This suggests that DDDM, being simple and less resource-intensive, could serve as a practical screening tool in busy, high-burden laboratories. Nevertheless, BDEM remains the CLSI-endorsed

method and is better suited for confirmatory testing. Consistent with the results, Harris H et al., showed that broth-based assays offer high reproducibility [12], while Taha R et al., demonstrated that disc-based approaches can provide reliable frontline screening, reinforcing the complementary role of both methods [7].

Clinical implications of the present findings include the potential utility of the CZA+ATM combination as a salvage therapy for MBL-producing CRE, particularly in regions with high prevalence of such resistance mechanisms. Future perspectives should focus on correlating in-vitro synergy results with patient outcomes to establish the clinical efficacy of this combination. In addition, molecular characterisation of resistance mechanisms is necessary to better understand non synergistic isolates and guide tailored therapy.

Limitation(s)

The limitation of the study included the evaluation of CRE isolates from samples received at a single centre, which does not necessarily resonate with the other hospitals in the geographical region. Genotypic methods were not employed to detect carbapenemase resistance genes or investigate coproduction of serine carbapenemases, ESBLs, or AmpC β-lactamases. In addition, as this was a descriptive, laboratory-based study that included all consecutive eligible isolates obtained during the study period, a formal sample size calculation was not performed. Also, our centre, being a tertiary care centre, recorded an elevated rate of CRE, as patients admitted or coming to our OPD were either referred patients from other smaller centres or those who were non compliant with the treatment they were receiving from them. Also, no data were available regarding the clinical utility of the CZA and ATM combination in this setting.

CONCLUSION(S)

The CRE pose a major therapeutic challenge in ICU patients. This study demonstrated high in-vitro synergy between CZA and ATM against MBL-producing CRE isolates. A strong concordance was observed between DDDM and BDEMs. The simpler disc-based method can serve as a reliable screening tool for synergy detection. Routine use of this combination may improve treatment outcomes in infections caused by MDR CRE.

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Authors' contribution: NY: Protocol development ,Conception and design; NY, CS, NT: Methodology used to collect isolates; KK: Data collection; CS, SSI, NT: Data analysis, Data curation; CS: Supervision; NY, CS : Writing original draft, Interpretation of Data; CS, SSP: Writing review and editing. All authors read and approved the final manuscript for submission.

REFERENCES

[1] De Pascale G, Cortegiani A, Rinaldi M, Antonelli M, Cattaneo S, Cecconi M, et al. Incidence of hospital-acquired infections due to carbapenem-resistant Enterobacterales and *Pseudomonas aeruginosa* in critically ill patients in Italy: A multicentre prospective cohort study. Crit Care. 2025;29(1):32.
[2] Sharma K, Tak V, Nag VL, Bhatia PK, Kothari N. An observational study on carbapenem-resistant Enterobacterales colonisation and subsequent risk of infection in an adult intensive care unit at a tertiary care hospital in India. Infect Prev Pract. 2023;5(4):100312. Doi: 10.1016/j.infpip.2023.100312. PMID:37868258; PMCID:PMC10585280.
[3] Van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence. 2017;8(4):460-69.
[4] Palzkill T. Metallo-β-lactamase structure and function. Ann N Y Acad Sci. 2013;1277(1):91-104.
[5] Karlowsky JA, Kazmierczak KM, de Jonge BLM, Hackel MA, Sahm DF, Bradford PA. In-vitro activity of aztreonam-avibactam against Enterobacteriaceae and *Pseudomonas aeruginosa* isolated by clinical laboratories in 40 countries from 2012 to 2015. Antimicrob Agents Chemother. 2017;61(7):e00472-17.
[6] Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America 2023 guidance on the treatment of antimicrobial resistant Gram-negative infections. Clin Infect Dis. 2023;ciad428.
[7] Taha R, Kader O, Shawky S, Rezk S. Ceftazidime-avibactam plus aztreonam synergistic combination tested against carbapenem-resistant Enterobacterales characterized phenotypically and genotypically: A glimmer of hope. Ann Clin Microbiol Antimicrob. 2023;22(1):21.
[8] Jayol A, Nordmann P, Poirel L, Dubois V. Ceftazidime/avibactam alone or in combination with aztreonam against colistin-resistant and carbapenemase-producing *Klebsiella pneumoniae*. J Antimicrob Chemother. 2020;75:1707-13.
[9] Sahu C, Pal S, Patel SS, Singh S, Gurjar M, Ghoshal U. Phenotypic synergy testing of ceftazidime-avibactam with aztreonam in a university hospital having high number of metallo-β-lactamase producing bacteria. Infect Dis (Lond). 2020;52(11):801-07. Doi: 10.1080/23744235.2020.1788224. PMID:32628575.
[10] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disc susceptibility tests. 34th ed. CLSI standard M100. Wayne (PA): CLSI; 2024.
[11] Khan S, Das A, Vashisth D, Mishra A, Vidyarthi AJ, Gupta R, et al. Evaluation of a simple method for testing aztreonam and ceftazidime-avibactam synergy in New Delhi metallo-beta-lactamase producing Enterobacterales. PLoS One. 2024;19(5):e0303753.
[12] Harris H, Tao L, Jacobs EB, Bergman Y, Adebayo A, Tekle T, et al. Multicenter evaluation of an MIC-based aztreonam and ceftazidime-avibactam broth disk elution test. J Clin Microbiol. 2023;61(5):e01647-22.
[13] Hu W, Li M, Lu W, Guo S, Li J. Evaluation of MASTDISCS combi Carba plus for the identification of metallo-β-lactamases, KPC and OXA-48 carbapenemase genes in Enterobacteriaceae clinical isolates. Lett Appl Microbiol. 2020;70(1):42-47.
[14] Aiesh BM, Maali Y, Qandeel F, Omarya S, Taha SA, Sholi S, et al. Epidemiology and clinical characteristics of patients with carbapenem-resistant Enterobacterales infections: Experience from a large tertiary care center in a developing country. BMC Infect Dis. 2023;23(1):644.
[15] Palacios-Baena ZR, Giannella M, Manissero D, Rodríguez-Baño J, Viale P, Lopes S, et al. Risk factors for carbapenem-resistant Gram-negative bacterial infections: A systematic review. Clin Microbiol Infect. 2021;27(2):228-35.
[16] Rajni E, Duggal S, Gajjar D, Sharma R, Garg V, Khatri PK. First report of molecular epidemiology of carbapenem-resistant Enterobacteriaceae from a tertiary level hospital in Rajasthan, Western India. J Med Sci. 2022;8(3):200.
[17] Prabhala S, Sundaresan A, Varaiya A. Prevalence and molecular characterization of carbapenem-resistant Gram-negative bacilli in a tertiary care hospital in Mumbai. IP Int J Med Microbiol Trop Dis. 2023;3:150-54.
[18] Sumangala B. Detection of carbapenem-resistant Enterobacteriaceae from various clinical samples: A record-based study in a tertiary care hospital in Mandya. Indian J Microbiol Res. 2023;10(2):86-90.
[19] Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasevic´ AT, et al. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): A prospective, multinational study. Lancet Infect Dis. 2017;17(2):153-63.
[20] Kotb S, Lyman M, Ismail G, Abd El Fattah M, Girgis SA, Etman A, et al. Epidemiology of carbapenem-resistant Enterobacteriaceae in Egyptian intensive care units using national healthcare-associated infections surveillance data, 2011-2017. Antimicrob Resist Infect Control. 2020;9(1):2.
[21] Sader HS, Carvalhaes CG, Arends SJR, Castanheira M, Mendes RE. Aztreonam/avibactam activity against clinical isolates of Enterobacterales collected in Europe, Asia and Latin America in 2019. J Antimicrob Chemother. 2020;76(3):659-66.
[22] Mittal S, Mamoria VP, Singla A, Bacchani D, Chaudhary P. Phenotypic detection of metallo-β-lactamase in carbapenem-resistant clinical isolates of *Klebsiella pneumoniae* ssp. pneumoniae at a tertiary care hospital in Jaipur. J Med Sci Clin Res. 2020;8:703-08.
[23] Sreenivasan P, Sharma B, Kaur S, Rana S, Biswal M, Ray P, et al. In-vitro susceptibility testing methods for the combination of ceftazidime-avibactam with aztreonam in metallo-beta-lactamase producing organisms: Role of combination drugs in antibiotic resistance era. J Antibiot (Tokyo). 2022;75:454-62.
[24] Vázquez-Ucha JC, Alonso-Garcia I, Guijarro-Sánchez P, Lasarte-Monterrubio C, Álvarez-Fraga L, et al. Activity of aztreonam in combination with novel β-lactamase inhibitors against metallo-β-lactamase-producing Enterobacterales from Spain. Int J Antimicrob Agents. 2023;61:106738.

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