Determination of mCIM and eCIM production and susceptibility pattern of ***Carbapenemase*** producing ***Enterobacterales*** and ***Pseudomonas aeruginosa*** against Ceftriaxone-Sulbactam with Disodium EDTA

INTRODUCTION:

Antimicrobial resistance (AMR) has emerged as a significant global health threat, with *Carbapenemase producing Enterobacterales (CPE) and Carbapenemase producing Pseudomonas aeruginosa* posing serious treatment challenges in clinical settings. These multidrug-resistant (MDR) / Pan drug Resistant (PDR) pathogens are commonly associated with healthcare-associated infections, pneumonia, skin and soft tissue infections and urinary tract infections. The increasing prevalence of carbapenem resistance has rendered conventional treatment options ineffective, necessitating the exploration of alternative combination therapeutic strategies like Ceftazidime-Avibactam (CAZ-AVI) and other BL-BLI combinations(1,2).

Ceftazidime-Avibactam a third-generation cephalosporin with a β-lactamase inhibitor, effectively targets KPC, OXA-48, and certain AmpC-producing strains. However, this combination lacks efficacy against Metallo-β-lactamase (MBL) producers, such as NDM-1. Ceftriaxone-Sulbactam with Disodium EDTA offers a novel approach by incorporating EDTA, a potent metal chelator that neutralizes MBL enzymes by disrupting their zinc-dependent activity. This combination shows promising strategy in overcoming resistance mechanisms seen in MBL-producing bacteria. Accurate susceptibility testing is crucial in guiding clinicians toward appropriate treatment strategies. Evaluating the efficacy of these antibiotic combinations against Carbapenem Resistant *Enterobacterales (CRE)* and Carbapenem Resistant *Pseudomonas aeruginosa* (CRPA) will provide valuable insights for improved therapeutic outcomes and infection control practices. Thus, this study aims to assess the mCIM and eCIM production among CRE and CRPA isolates and to compare the susceptibility patterns of Ceftriaxone-Sulbactam with Disodium EDTA combination against Ceftazidime-avibactam(3–6).

MATERIALS AND METHODS

Study Strategy and Setting

This prospective observational study was carried out in the Dept. of Microbiology at Mahatma Gandhi Medical College and Research Institute (MGMCRI), SBV, Puducherry, India, over a period of three months from May to July 2022. The study focused on clinical isolates of *Enterobacterales* and *Pseudomonas aeruginosa* exhibiting resistance to carbapenem antibiotics.

Isolation and Bacterial Strains Identification

Consecutive, non-duplicative, clinical isolates of CRE and CRPA procured from various clinical specimens (e.g., urine, pus, respiratory secretions) were submitted to this study. Identification of the isolates was executed using standard biochemical tests as per standard laboratory operating protocol (ICMR guidelines on sample processing)(7).

Screening for Carbapenem Resistance

Screening on the initial basis for carbapenem resistance was performed using meropenem (10 µg) and imipenem (10 µg) discs on the Mueller-Hinton agar (MHA) following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates showing intermediate or resistance to carbapenem were subjected to additional confirmatory tests.

Modified Carbapenem Inactivation Method (mCIM):

The mCIM was perform as per CLSI guidelines to distinguish Carbapenemase production. A 1 µL loopful of *Enterobacterales* or 10 µL loopful of *P. aeruginosa* was suspended in 2 mL of tryptic soy broth (TSB) from an overnight growth from blood agar. The suspension was vortexed for 10–15 seconds. A 10-µg meropenem disc was added to the TSB tube ensuring full submersion and it was incubated at 35 ± 2°C for 4 hours and 15 minutes. Meanwhile, a 0.5 McFarland standard suspension of *Escherichia coli* ATCC Strain 25922 was prepared in sterile saline. A Mueller-Hinton agar plate was inoculated with the *E. coli* suspension to create a lawn culture and allowed to dry for 3–10 minutes. Following incubation, the meropenem disc was removed from the TSB using a sterile 10-µL loop and placed onto the *E. coli* lawn. Plates were incubated at 35 ± 2°C in ambient air for 18–24 hours, and zone diameters were interpreted conferring to the CLSI guidelines.

Modified – EDTA Carbapenem Inactivation Method (eCIM):

(To differentiate Metallo-β-lactamase (MBL) producers)

A separate TSB tube was prepared for each isolate as described in mCIM, with the addition of 20 µL of 0.5 M EDTA to achieve a final concentration of 5 mm The same protocol as mCIM was followed. An increase in the zone diameter by ≥5 mm in eCIM compared to mCIM indicated MBL production(8–10).

Antimicrobial Susceptibility Testing

Antibiotic susceptibility to ceftriaxone–sulbactam–EDTA (CSE) combination and ceftazidime–avibactam was estimated using the Kirby-Bauer Method (disk diffusion) on MHA plates as per CLSI standards. Plates were incubated at 35 ± 2°C for 16–18 hours. Zone diameters were calculated and interpreted using "CLSI 2022 – M100" criteria or manufacturer-specified breakpoints for investigational agents where applicable.

RESULTS

Demographic Characteristics of Patients with Carbapenem-Resistant Infections:

A total of 110 carbapenem-resistant isolates were recovered during the study period. The age and gender distribution of the affected patients is depicted in Figure 2. Most resistant isolates were observed in patients aged >60 years, with female predominance (22) followed by males (15). Similarly, between 46–60 years and 26-35 years of age also, majority were females.

Antibiotic Susceptibility Profile of Carbapenem-Resistant Isolates

The susceptibility profile of the isolates revealed complete resistance (100%) to five major antibiotics: imipenem, meropenem, ampicillin, ceftriaxone, and ciprofloxacin. Variable levels of sensitivity were observed for other antibiotics. Gentamicin showed the highest sensitivity (57%), followed by Cefoperazone–sulbactam (50%) and cotrimoxazole (25%). Sensitivity to amikacin and piperacillin–tazobactam was low (23%, 34% respectively).

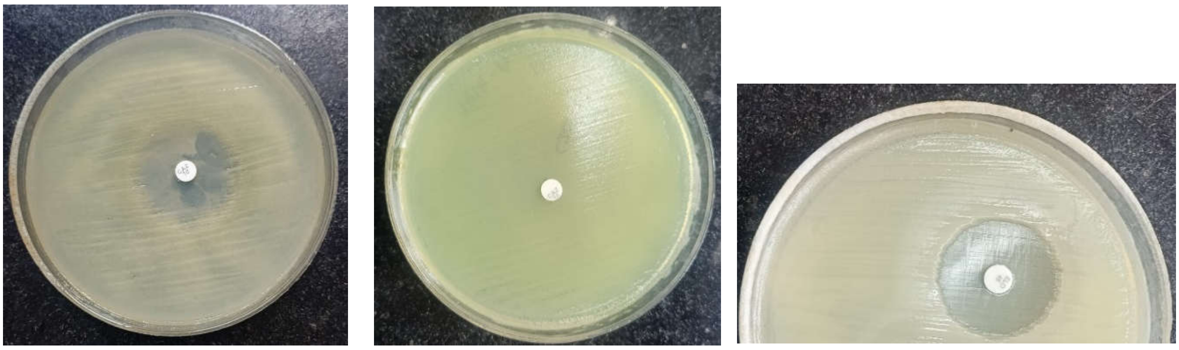

FIG 1: Ceftazidime – Avibactam (CAZ-AVI - 10µg) sensitive & ceftazidime - avibactam resistance & Ceftriaxone–Sulbactam–EDTA (20µg) sensitive

Table 1: Antibiotic resistance profile of carbapenem-resistant isolates

Antibiotic	Resistant (n, %)	Sensitive (n, %)
Imipenem	110 (100%)	0 (0%)
Meropenem	110 (100%)	0 (0%)
Ampicillin	110 (100%)	0 (0%)
Ceftriaxone	110 (100%)	0 (0%)
Ciprofloxacin	110 (100%)	0 (0%)
Cotrimoxazole	83 (75%)	27 (25%)
Amikacin	84 (76%)	26 (23%)
Piperacillin + Tazobactam	57 (66%)	53 (34%)
Cefoperazone + Sulbactam	55 (50%)	55 (50%)
Gentamicin	47 (43%)	63 (57%)

Carbapenemase Detection via mCIM and eCIM

Among the 110 carbapenem-resistant isolates, 29% were positive for Metallo-β-lactamase (MBL) and 12% were found to produce serine Carbapenemase production. Notably, 59% of isolates did not show Carbapenemase activity via mCIM/eCIM, indicating alternative resistance mechanisms (Fig 3).

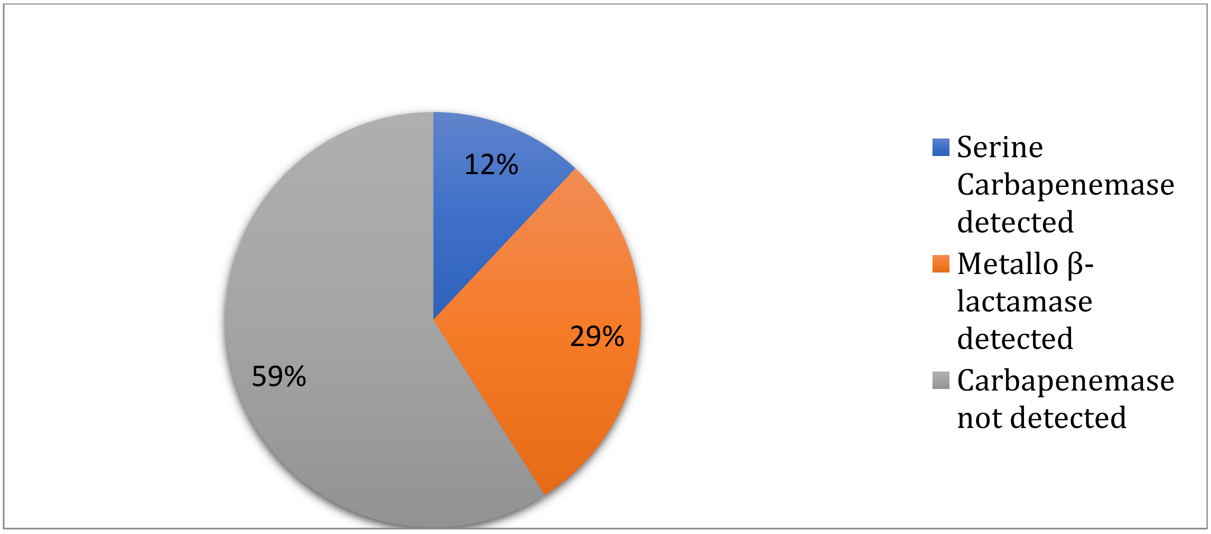

Figure 3: Distribution of Carbapenemase production by mCIM and eCIM

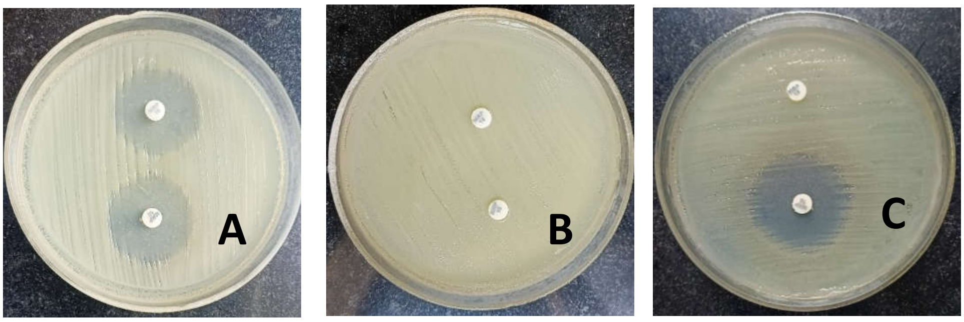

Figure 4: A. Carbapenemase not detected, B. Serine Carbapenemase detected C. Metallo beta- lactamases detected

Sample-wise Distribution of Carbapenemase Producers

The prevalence of different types of Carbapenemase varied across clinical sample types. Respiratory samples had the highest rate of serine Carbapenemase detection (19%), whereas urine samples showed the highest MBL detection (39%). Carbapenemase was not detected in over half of the isolates in each group (Table 2).

Table 2: Sample-wise distribution of mCIM and eCIM findings:

Carbapenemase Type	Exudates (n=24)	Urine (n=33)	Respiratory (n=53)
Metallo-β-lactamase detected	7 (29%)	13 (39%)	12 (23%)
Serine Carbapenemase detected	1 (4%)	2 (6%)	10 (19%)
Carbapenemase not detected	16 (67%)	18 (55%)	31 (58%)

Susceptibility to Ceftriaxone–Sulbactam + Disodium EDTA:

Ceftriaxone–sulbactam combined with disodium EDTA demonstrated significantly higher susceptibility pattern, with an overall sensitivity of 81% (89/110). Notably, none of the exudate isolates were resistant, while only 5% of urine and 15% of respiratory isolates showed resistance (Table 3).

Table 3: Ceftriaxone–Sulbactam + Disodium EDTA susceptibility pattern

Sample Type	Resistant (n, %)	Sensitive (n, %)
Exudates	0 (0%)	25 (23%)
Urine	5 (5%)	24 (22%)
Respiratory	16 (15%)	40 (35%)
Total	21 (19%)	89 (81%)

Susceptibility to Ceftazidime–Avibactam

Ceftazidime–Avibactam showed an overall sensitivity of 26% (28/110) across all sample types. The highest resistance was detected in respiratory isolates (42%, 46/110). None of the exudate or urine isolates were found to be sensitive (Table 4).

Table 4: Ceftazidime–Avibactam susceptibility pattern

Sample Type	Resistant (n, %)	Sensitive (n, %)
Exudates	19 (17%)	0 (0%)
Urine	17 (15%)	0 (0%)
Respiratory	46 (42%)	28 (26%)
Total	82 (74%)	28 (26%)

DISCUSSION:

The increasing burden of carbapenem-resistant Enterobacterales (CRE) and *Pseudomonas aeruginosa* (CRPA) in clinical settings has posed a formidable challenge to antimicrobial therapy and infection control. The complete resistance observed in all 110 clinical isolates to frontline antibiotics such as Imipenem, Meropenem, Ceftriaxone, and Ciprofloxacin underscores the grave limitations of current treatment options. In this context, accurate phenotypic detection and evaluation of novel combination therapies become indispensable(11–13).

The modified Carbapenem inactivation method (mCIM) and EDTA-Carbapenem inactivation method (eCIM) employed in this study demonstrated robust phenotypic discrimination between serine Carbapenemase and Metallo-β-lactamases (MBLs), consistent with findings by Verma et al. (2024) and Tsai et al. (2020)(3,14). Our data revealed MBL production in 29% of isolates and serine Carbapenemase activity in 12%, while a significant 59% of isolates were negative by both mCIM and eCIM. This finding echoes the observations of Aboulela et al. (2023), who reported the presence of Carbapenemase-encoding genes in isolates that yielded negative phenotypic results. These discrepancies highlight the limitations of mCIM/eCIM in detecting low-level or heterogeneously expressed Carbapenemase and emphasize the need for adjunct molecular diagnostics(15).

Interestingly, Lasko et al. (2020) noted that eCIM sensitivity may vary with the type of Carbapenemase and that increasing EDTA concentration could enhance detection, especially for IMP-producing P. aeruginosa. This aligns with our observation that MBL detection was higher in urine-derived isolates, a niche where biofilm production and trace enzymatic activity may mask conventional phenotypic positivity. Thus, phenotypic methods such as mCIM and eCIM, although practical and cost-effective, may require methodological refinement or supplementation with PCR-based assays for optimal diagnostic performance(4).

On the therapeutic front, the comparative evaluation of ceftriaxone–sulbactam + disodium EDTA (CSE) and ceftazidime–avibactam (CAZ-AVI) revealed crucial insights. The superior in vitro efficacy of CSE (81% sensitivity) over CAZ-AVI (26%) in our study reflects its potent activity, particularly against MBL-producing isolates. EDTA, a known chelator of divalent cations, effectively disrupts zinc-dependent MBL activity, thereby restoring β-lactam susceptibility. This is particularly relevant in the Indian setting, where NDM-type MBLs predominate and where agents like CAZ-AVI, which lack activity against class B β-lactamases, offer limited utility (Verma et al., 2024)(14).

Conversely, CAZ-AVI showed poor performance, especially among respiratory isolates, corroborating Lasko et al. (2020)(4), who documented CAZ-AVI's limited spectrum against non-KPC Carbapenemase. This poor performance also underscores the geographic variability in resistance mechanisms and highlights the inadequacy of universal empirical therapy. The high rate of CSE susceptibility in respiratory isolates (35%) and complete susceptibility among exudate-derived isolates reinforces the clinical value of EDTA-based combinations in site-specific infections.

The demographic analysis points to a higher prevalence of CRE/CRPA among elderly females, likely due to comorbidities, increased catheter use, and anatomical susceptibility to Urinary Tract Infections. Such trends underline the importance of stratified risk assessment in hospital infection control policies.

Clinical implications of this study are significant. First, the practical utility of mCIM and eCIM in routine diagnostic laboratories remains high, particularly in low-resource settings, despite their limitations. Second, CSE emerges as a promising, cost-effective therapeutic alternative in MBL-endemic regions. However, resistance to CSE (19%) warrants cautious use and supports the need for antibiotic stewardship. Third, the failure of CAZ-AVI in most cases stresses the importance of resistance mechanism-guided therapy rather than blind empirical application of newer antimicrobials.

To maximize clinical impact, integrating phenotypic methods with rapid molecular diagnostics can enhance diagnostic accuracy and guide tailored therapy. Furthermore, as resistance mechanisms diversify, therapeutic innovation must keep pace. The development of next-generation β-lactam/β-lactamase inhibitor combinations targeting MBLs or alternative non-β-lactam agents may be pivotal in future antimicrobial strategies.

Future research should focus on: Future research should prioritize a multifaceted approach to effectively combat the increasing risk of multidrug-resistant organisms (MDROs). A key focus should be on **multicentric surveillance studies** to capture regional and temporal variations in resistance patterns, enabling more informed empirical treatment protocols. Additionally, **clinical outcome-based validation** of novel antibiotic combinations is essential to establish their real-world efficacy and safety, beyond in vitro susceptibility data. The practice of **whole-genome sequencing (WGS)** can provide comprehensive insights into the genetic makeup of resistant isolates, facilitating the identification of resistance genes, mobile genetic elements, and clonal relationships. Furthermore, there is a pressing need to explore **synergistic combination therapies**, which may enhance antimicrobial activity, reduce resistance emergence, and improve patient outcomes. Together, these research directions will support evidence-based antimicrobial stewardship and inform the development of next-generation treatment strategies.

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