Original Article

DOI: 10.7860/JCDR/2026/84798.23983

Microbiology Section

Antimicrobial Susceptibility Profile of Salvage Antibiotics against WHO Priority Carbapenem-resistant KAPE Pathogens using CLSI Recommended Reference Methods: A Laboratory-based Cross-sectional Study

PRERNA S SALIAN[1], ARUNA POOJARY[2], ANURAG KUMAR BARI[3]

ABSTRACT

Introduction: Increasing reliance on salvage antibiotics for Carbapenem-Resistant Gram-Negative (CR-GNB) infections necessitates updated, locally generated in-vitro susceptibility data using standardised laboratory methods to guide rational antimicrobial therapy.

Aim: To evaluate the in-vitro antimicrobial susceptibility profile of salvage antibiotics against carbapenem-resistant *Klebsiella pneumoniae* (CRKP), *Acinetobacter baumannii* (CRAB), *Pseudomonas aeruginosa* (CRPA) and *Escherichia coli* (CREC), collectively termed CR-KAPE.

Materials and Methods: This laboratory-based cross-sectional study was conducted at the Department of Microbiology, Breach Candy Hospital Trust, Mumbai, Maharashtra, India from January 2022 to December 2024. A total of 366 consecutive, non-duplicate CR-KAPE isolates from various clinical specimens (blood, Lower-Respiratory Tract (LRT), Intra-abdominal Infection (IAI), skin and soft-tissue, urine) were included as per predefined inclusion criteria. Identification and confirmation of CR were performed using conventional methods and the VITEK 2 system. Antimicrobial Susceptibility Testing (AST) of salvage antibiotics was carried out using broth microdilution, agar dilution, and E-test-Disk diffusion assay, interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Demographic details were recorded, and data were analysed as descriptive statistics.

Results: Among 366 CR-KAPE isolates, 138 (37.7%) were CRKP, 88 (24.0%) CRAB, 57 (15.6%) CRPA, and 83 (22.7%) CREC. Tigecycline susceptibility was observed in 52/72 (72.2%) CRAB and 81/83 (97.5%) CREC isolates. Colistin intermediate susceptibility ranged from 88% to 98% across CR-KAPE isolates, while polymyxin B demonstrated higher intermediate susceptibility rates (93% in CRKP and 98%-100% in other isolates). In 45.1% (165/366) of isolates, the MIC of polymyxin B was lower than that of colistin, while in 39.6% (145/366) it was similar. CRAB demonstrated 100% (88/88) susceptibility to CSE at a fixed EDTA concentration of 4740 μg/mL. Fosfomycin susceptibility among CREC urine isolates was 38/42 (90%). Ceftazidime-Avibactam (CZA)-aztreonam synergy was observed in 108/137 (78.8%) CRKP and 45/75 (60.0%) CREC isolates.

Conclusion: The findings highlight the declining efficacy of available salvage antibiotics against CR-KAPE pathogens and emphasise the need for new antimicrobial agents targeting GNB infections. Rapid standardised methods and continuous surveillance are essential to preserve last-resort drugs and guide effective treatment strategies.

Keywords: Antimicrobial susceptibility testing, Bacterial, Drug resistance, Gram-negative bacteria, Multidrug resistance

INTRODUCTION

Antimicrobial Resistance (AMR) remains a major global public health threat, with an estimated 8.22 million associated deaths projected annually by 2050 worldwide. CR-GNB is a significant contributor to this burden, substantially increasing mortality rates. Globally, deaths associated with CR-GNB infections increased from approximately 619,000 in 1990 to over 1.03 million in 2021 [1]. Among these, CRKP, CRAB, CRPA and CREC collectively termed as CR-KAPE, pose a serious threat and are listed in the Indian Priority Pathogens List (IPPL, 2021) [2]. The World Health Organisation (WHO) 2024 further classifies CRKP, CREC and CRAB as critical priority pathogens and CRPA as high priority due to high mortality, morbidity and treatment challenges [3].

Carbapenems are often the drugs of choice for Multidrug-Resistant (MDR) infections. However, due to rising incidence of resistance in GNB the efficacy of carbapenems is diminishing. In addition, treatment failures can also occur due to heterogeneous resistance mechanisms and high inoculum effects. CR is primarily mediated by the production of carbapenemases including KPC, NDM, OXA-type, and VIM enzymes, along with additional mechanisms such as efflux pump overexpression, porin loss, and biofilm formation. These diverse and often co-existing mechanisms contribute to variable resistance phenotypes, complicating both detection and therapeutic decision-making. Furthermore, infections caused by CR organisms are frequently healthcare-associated and associated with limited treatment options, often necessitating the use of salvage agents and individualised therapy guided by in-vitro susceptibility testing [4]. This growing burden is reflected in both global and Indian data, with CRKP prevalence reported as high as 67-68% [5,6], and CRAB demonstrating alarmingly high resistance levels, in some settings approaching near-universal prevalence [7,8]. In contrast, CREC exhibits considerable variability across regions [5,6,8], whereas data on CRPA prevalence remain limited in the Indian context, underscoring the substantial impact of CR-KAPE infections on patient outcomes.

The persistence of CR-KAPE in the updated WHO priority list in 2024 clearly indicates the urgent need, not only for new antimicrobials but also for strategic antimicrobial stewardship interventions and a better understanding of the carbapenemase profiles for guiding targeted therapy. With emergence of CR, treatment increasingly relies on salvage antibiotics such as polymyxins, newer β-lactam/β-lactamase inhibitors (e.g., imipenem-relebactam, meropenem-vaborbactam), CZA, fosfomycin, newer tetracycline-derivatives (eravacycline and omadacycline), and siderophore cephalosporins like cefiderocol [9]. However, in the Indian setting, the availability of many of these agents remains limited, with CZA and fosfomycin being among the few widely accessible options.

Given the increasing reliance on salvage antibiotics for the management of CR-KAPE infections, locally generated susceptibility data using CLSI -recommended reference methods are important to inform therapeutic decision-making and implement antimicrobial stewardship practices. However, data on the susceptibility profiles of salvage antimicrobial agents from India are scarce, particularly based on CLSI standards. This is further compounded by gaps in microbiological infrastructure and surveillance systems in low- and middle-income settings, with limited availability of data linking microbiological findings to clinical outcomes, resulting in fragmented and non-representative evidence [1]. Variations in testing methodologies and local resistance patterns further limit the generalisability of existing findings. This represents a critical gap in the current literature, with direct implications for clinical decision-making and antimicrobial stewardship. Additionally, the lack of standardised antimicrobial susceptibility testing practices across laboratories and limited integration of microbiological data into clinical decision-making further hinder effective antimicrobial stewardship in the Indian setting.

Therefore, this study aimed to evaluate the antimicrobial susceptibility profiles of available salvage antibiotics against CR-KAPE isolates using CLSI-recommended reference methods, to generate clinically relevant data for guiding targeted therapy and antimicrobial stewardship in the context of rising CR.

MATERIALS AND METHODS

This laboratory-based cross-sectional study was conducted in the Department of Microbiology, Breach Candy Hospital Trust, Mumbai, Maharashtra, India, from January 2022 to December 2024. The study was approved by the Research Advisory Board and Ethics Committee of Breach Candy Medical Research Centre (BCMRC) (Project Nos. - P2/2022 and P9-2021). As the study involved bacterial isolates obtained as part of routine diagnostic services, with no patient identifiers or clinical intervention, the requirement for informed consent was waived by the Ethics Committee. All non-duplicate CR-KAPE isolates fulfilling the inclusion criteria during the study period were included (complete enumeration). A total of 366 isolates were analysed.

Inclusion criteria: Non-duplicate clinical isolates of CR-KAPE from various clinical specimens including urine, blood, IAI, and LRT samples, during the study period were included in the study.

Exclusion criteria: Duplicate isolates from the same patient and isolates for which prior AST data for salvage antibiotics were already available were excluded from the study.

Study Procedure

All isolates were subcultured onto 5% Sheep Blood Agar (SBA) (bioMérieux, France) and MacConkey agar (BD, USA), incubated at 35±2°C for 18-20 hours. Preliminary identification (ID) was performed based on colony morphology and conventional biochemical tests, including Simmon's citrate agar, urea agar base, and Triple Sugar Iron (TSI) agar (HiMedia, India), along with indole production using Kovac's reagent (HiMedia, India) and the oxidase test (HiMedia, India). Final ID was confirmed using the VITEK 2 Compact system (bioMérieux, France) with GN-ID cards, following the manufacturer's instructions. QC was maintained throughout the study period using *Escherichia coli* ATCC 25922, *Escherichia coli* NCTC 13846 and *Pseudomonas aeruginosa* ATCC 27853 (bioMérieux, France).

Phenotypic testing: CR was primarily screened using both VITEK 2 and Disk-Diffusion (DD). A standard 0.5 McF inoculum was prepared from overnight cultures. For automated AST, the suspension was processed on VITEK 2 using N-405 cards for fermenters and N-406 cards for non-fermenters, as per the manufacturer's instructions.

The same 0.5 McF suspension was used for DD testing. Lawn culture was performed on BBL™ Mueller–Hinton II (MH-II) agar (BD, USA), and disks of meropenem (MRP, 10 µg), imipenem (IPM, 10 µg), and ertapenem (ETP, 10 µg) (Microxpress, India) were applied. Plates were incubated aerobically at 35±2°C for 16-18 hours. QC was performed using the reference strains mentioned above.

Interpretation of zone diameters for DD and Minimum Inhibitory Concentration (MIC) values generated by VITEK 2 was carried out according to CLSI guidelines [10]. Carbapenem breakpoints used for interpretation are presented in [Table/Fig-1].

Genotypic testing: Carbapenemase genes were confirmed using the TRU-RAPID® O.K.N.V.I. RESIST-5 Rapid Test [11] (OXA-48, KPC, NDM, VIM and IMP) (3BLF0019-25) (3B BlackBio Dx Limited, India) performed on fresh subcultures, following the manufacturer's instructions. Results were interpreted qualitatively, with the presence of a specific visible band indicating a positive result and absence indicating a negative result for the corresponding carbapenemase gene. For blood culture isolates, the BioFire® FilmArray® Blood Culture Identification 2 (BCID2) Panel (RFIT-ASY-0147) (bioMérieux, France) was performed on blood cultures that flagged positive as part of routine diagnostic workflow, when requested by the treating clinician. Carbapenemase genes were reported as "detected" or "not detected" as per the manufacturer's instructions, and BCID2 results, when available, were included for analysis irrespective of parallel testing by TRU-RAPID® kits.

Broth Microdilution (BMD)

Polymyxins: BMD plates for Colistin (COL) and Polymyxin B (POL) were prepared using their sulphate salts (Sigma, USA) in two-fold dilutions, dissolved in BBL™ Cation-Adjusted Mueller-Hinton Broth (CAMHB) (BD, USA), as per CLSI M07 [12]. Final concentration range: 0.06–32 µg/mL.

Organism Type	Antibiotic (disk potency)	Susceptible (mm)	Intermediate (mm)	Resistant (mm)	CLSI guideline*
Enterobacterales	Imipenem (10 µg)	≥23	20-22	≤19	2022-2024
	Meropenem (10 µg)	≥23	20-22	≤19	2022-2024
	Ertapenem (10 µg)	≥22	19-21	≤18	2022-2024
Pseudomonas aeruginosa	Imipenem (10 µg)	≥19	16-18	≤15	2022-2024
	Meropenem (10 µg)	≥19	16-18	≤15	2022-2024
Acinetobacter spp.	Imipenem (10 µg)	≥22	19-21	≤18	2022-2024
	Meropenem (10 µg)	≥18	15-17	≤14	2022-2024

[Table/Fig-1]: CLSI breakpoints used for CR screening.
*CLSI guidelines were referred annually according to the study period; breakpoints remained unchanged from 2022 to 2024

CSE: In-house BMD plates for CSE (CTR/SBT/EDTA) were prepared using ceftriaxone (CTR) disodium salt hemi-heptahydrate, Sulbactam (SBT) Sodium CRS and Disodium Edetate (EDTA) CRS (Sigma, USA). CTR was serially diluted (0.125–64 µg/mL) with fixed SBT (4 µg/mL) & EDTA (4740 µg/mL, corrected for 10 mM) [13,14].

Plates (96-well, Tarsons, India) were inoculated with bacterial suspension of 5×10^5 CFU/mL, sealed and incubated at 35 ± 2°C for 18-20 hours. Results were interpreted as per CLSI [10]. COL and POL was performed for all CR-KAPE; CSE testing was limited to CRKP, CRAB and CREC as CTR is not recommended for *P. aeruginosa* [10].

Synergy testing E-test-Disk diffusion assay: The E-test strip of CZA (bioMérieux, France) and aztreonam (AT) (30 µg) disk (MicroXpress, India) were used to assess synergy in CREC and CRKP. A 0.5 McF suspension was lawn-cultured on MH-II (BD, USA). After drying, the CZA strip (0.016-256 µg/mL with fixed 4 µg/mL avibactam) was placed centrally and AT disk 15 mm away near CZA breakpoint (8 µg/mL). Plates were incubated at 37°C for 16-18 hours. QC was performed using *Escherichia coli* ATCC 25922.

Synergy interpretation used two approaches [15]:

1. Qualitative: Presence of an inverted D-shaped zone of inhibition indicates synergy.
2. Quantitative: The zone radius of AT disc alone was measured, and MIC for CZA was interpreted based on CLSI [10] to assess synergy.

Agar Dilution (AD): In-house fosfomycin AD plates were prepared to determine the MIC values for CREC urine isolates. MHA plates supplemented with 25 µg/mL of glucose-6-phosphate (Sigma, USA) and fosfomycin (Sigma, USA) at concentrations ranging from 0.125-512 µg/mL were used [16]. A bacterial suspension adjusted to a 0.5 McF was diluted to 10^6 CFU/mL and then dispensed onto 20 mL agar plates to achieve a final inoculum of 10^4 CFU/mL, as recommended by CLSI M07 [12]. Plates were incubated at 37°C for 16-18 hours and interpreted according to CLSI [10].

STATISTICAL ANALYSIS

All data were entered into Microsoft Excel 2021 (Microsoft Corp., Redmond, WA, USA) and analysed using descriptive statistics, expressed as numbers and percentages.

RESULTS

The study included 366 clinical CR-KAPE isolates, comprising CRKP 138 (37.7%), CRAB 88 (24%), CRPA 57 (15.6%), and CREC 83 (22.7%).

For CRKP and CREC, urine was the predominant source of isolation, followed by blood {CRKP: urine 51/138 (37%), blood 40/138 (29%); CREC: urine 45/83 (54.2%), blood 16/83 (19.3%)}. For CRAB, LRT was the major source 54/88 (61.4%), while for CRPA, isolates were obtained mainly from the LRT 20/57 (35%) and urine 18/57 (31.6%) [Table/Fig-2].

Clinical specimens	CRKP n (%)	CRAB n (%)	CRPA n (%)	CREC n (%)
Blood	40 (29)	8 (9.1)	7 (12.3)	16 (19.3)
IAI	10 (7.2)	10 (11.4)	0	11 (13.3)
LRT	22 (15.9)	54 (61.4)	20 (35)	1 (1.2)
Skin and Soft-tissue	15 (10.9)	6 (6.8)	1 (1.8)	7 (8.4)
Urine	51 (37)	4 (4.5)	18 (31.6)	45 (54.2)
Wound or Pus Swab	0	6 (6.8)	11 (19.3)	3 (3.6)
Total (n)	138	88	57	83

[Table/Fig-2]: Specimen distribution of CR-KAPE isolates.
Abbreviations: CRKP: Carbapenem-resistant *Klebsiella pneumoniae*; CRAB: Carbapenem-resistant *Acinetobacter baumannii*; CRPA: Carbapenem-resistant *Pseudomonas aeruginosa*; CREC: Carbapenem-resistant *Escherichia coli*; IAI: Intra-abdominal infections; LRT: Lower respiratory tract

The CRKP and CRAB isolates were predominantly Intensive Care Unit (ICU) associated {CRKP 66/138 (48%); CRAB 72/88 (82%)}. CRPA showed a mixed distribution with ICU 26/57 (45.6%) and Outpatients Department (OPD) 23/57 (40.4%) predominance. In contrast, CREC isolates were most frequently recovered from OPD 34/83 (41%), followed by Inpatients Department (IPD) 27/83 (32.5%) and ICUs 22/83 (26.5%) [Table/Fig-3].

Location	CRKP n (%)	CRAB n (%)	CRPA n (%)	CREC n (%)
OPD	40 (29)	4 (4.5)	23 (40.4)	34 (41)
IPD	32 (23)	12 (13.6)	8 (14)	27 (32.5)
ICU	66 (48)	72 (82)	26 (45.6)	22 (26.5)
Total (n)	138	88	57	83

[Table/Fig-3]: Location-wise distribution of CR-KAPE isolates.

The susceptibility (S) profile of CR-KAPE isolates is summarised in [Table/Fig-4], demonstrating negligible S to routinely used β-lactams across all organisms. Among CRKP, limited activity was observed only for gentamicin 24/134 (17.9%), amikacin 26/134 (19.4%), and trimethoprim-sulfamethoxazole 33/138 (24%). In CRAB, tigecycline demonstrated the highest activity with 52/72 (72%) susceptible isolates, while S to other tested agents remained low. CREC isolates retained S to tigecycline 81/83 (98%), amikacin 59/83 (71%), and gentamicin 54/83 (65%). These findings underscore the limited therapeutic options available for these pathogens.

Antibiotics	CRKP (n=138)	CRAB (n=88)	CRPA (n=57)	CREC (n=83)
Amoxicillin-clavulanate	0	-	-	0
Piperacillin-tazobactam	0	-	9 (16)	0
Cefoperazone/ sulbactam	-	-	-	0
Cefepime	5 (3.6)	1 (1.1)	10 (18)	-
Ceftriaxone	1 (0.7)	-	-	0
Ceftazidime	-	-	11 (19)	-
Cefuroxime	0	-	-	0
Aztreonam	-	-	-	-
Imipenem	0	-	0	0
Meropenem	0	-	0	0
Gentamicin	24/134 (17.9)*	1 (1.1)	-	54 (65)
Amikacin	26/134 (19.4)*	-	3/18 (17)*	59 (71)
Minocycline	-	12/66 (18)*	-	-
Tigecycline	8/118 (7)*	52/72 (72)*	-	81 (97.5)
Ciprofloxacin	0	1 (1.1)	6 (11)	0
Levofloxacin	-	4 (4.5)	5 (9)	-
Trimethoprim-sulfamethoxazole	33 (24)	6 (6.8)	-	19 (23)

[Table/Fig-4]: Susceptibility profile n (%) of CR-KAPE isolates to tested antibiotics.
CRKP: Carbapenem-resistant Klebsiella pneumoniae; CRAB: Carbapenem-resistant Acinetobacter baumannii; CRPA: Carbapenem-resistant Pseudomonas aeruginosa; CREC: Carbapenem-resistant Escherichia coli
$^{+}$0 indicates no susceptible isolates identified- indicates antibiotic not tested or not applicable for the respective organism; * denotes S n (%) for subset of isolates, reported as per automated system interpretation (VITEK 2 compact)

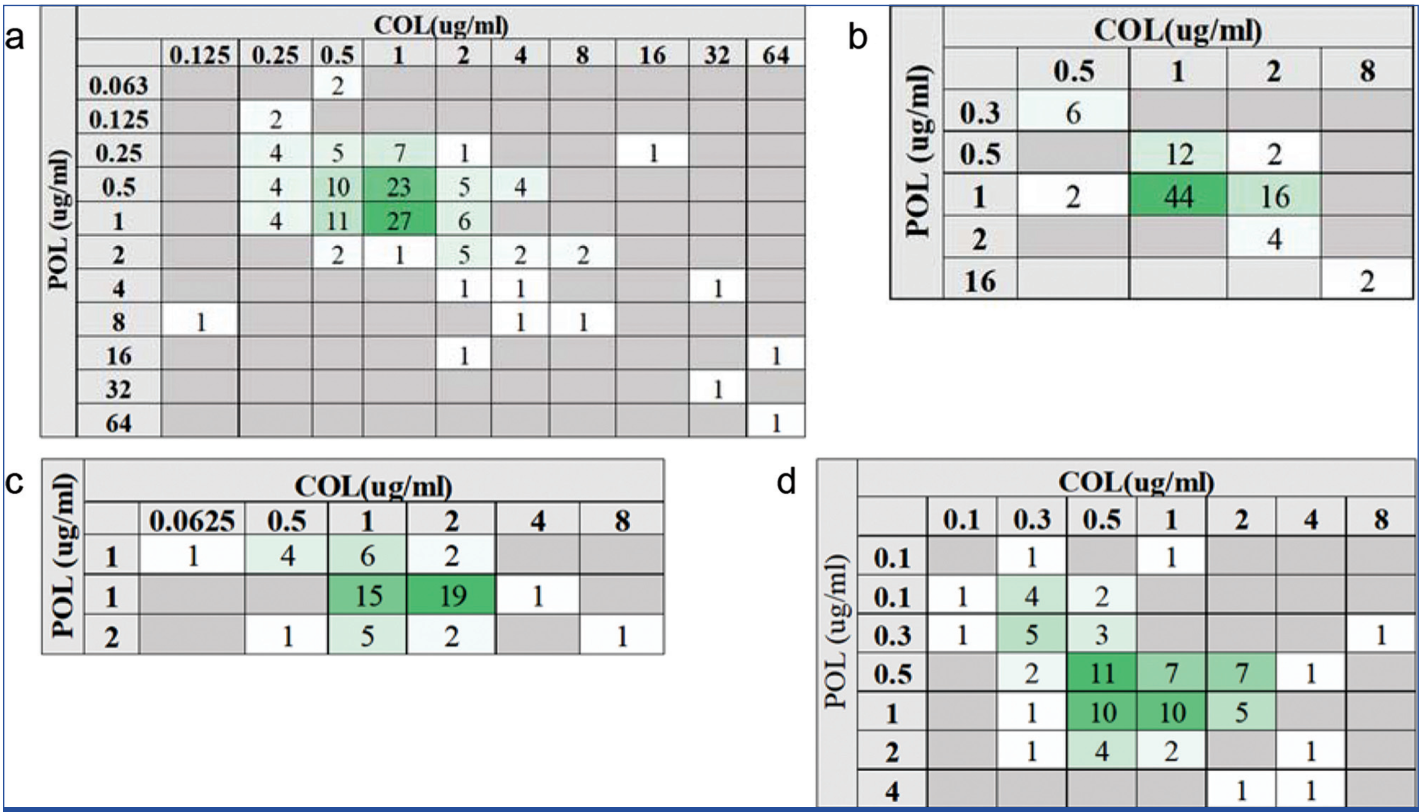
Regarding polymyxins, among CR-KAPE, CRKP showed the highest COL resistance (R) 16/138 (11.6%) and POL R% 10/138 (7.2%) [Table/Fig-5,6].

The CSE testing showed that CRAB (n=88) showed 100% S to CSE at 10 mM EDTA, with 40/88 (45.5%) isolates having MICs of 0.125/4 (CTR/SBT) and 36/88 (41%) having MICs of 0.25/4. In CRKP (n=99), 44/99 (44.4%) isolates were S, while CREC (n=48) showed 36/48 (75%) S [Table/Fig-7].

CZA-AT synergy was observed in 108/137 (78.8%) CRKP isolates and 45/75 (60%) CREC isolates [Table/Fig-8].

	CRKP		CRAB		CRPA		CREC	
MIC (µg/mL)	COL n (%)	POL n (%)	COL n (%)	POL n (%)	COL n (%)	POL n (%)	COL n (%)	POL n (%)
2	19 (13.8)	12 (8.7)	22 (25)	4 (4.5)	23 (40.4)	9 (15.8)	13 (15.7)	8 (9.6)
1	58 (42)	48 (35)	56 (63.6)	62 (70.5)	26 (45.6)	35 (61.4)	20 (24)	26 (31.3)
0.5	30 (21.7)	46 (33.3)	8 (9.1)	14 (15.9)	5 (8.7)	13 (22.8)	30 (36.1)	28 (33.7)
0.25	14 (10.2)	18 (13)	0	6 (6.8)	0	0	14 (17)	10 (12.1)
0.125	1 (0.72)	2 (1.4)	0	0	0	0	0	7 (8.4)
0.0625	0	2 (1.4)	0	0	1 (1.8)	0	2 (2.4)	2 (2.4)
Total n (%)	122 (88.4)	128 (92.8)	86 (97.7)	86 (97.7)	55 (96.5)	57 (100)	79 (95.2)	81 (97.5)

[Table/Fig-5]: MIC distribution of COL and POL for intermediate susceptible CR-KAPE isolates.



a

POL (ug/ml)	COL(ug/ml) 0.125	0.25	0.5	1	2	4	8	16	32	64
0.063			2							
0.125		2								
0.25		4	5	7	1			1		
0.5		4	10	23	5	4				
1		4	11	27	6					
2			2	1	5	2	2			
4					1	1			1	
8	1					1	1			
16					1					1
32									1	
64										1

b

POL (ug/ml)	COL(ug/ml) 0.5	1	2	8
0.3	6			
0.5		12	2	
1	2	44	16	
2			4	
16				2

c

POL (ug/ml)	COL(ug/ml) 0.0625	0.5	1	2	4	8
1	1	4	6	2		
1			15	19	1	
2		1	5	2		1

d

POL (ug/ml)	COL(ug/ml) 0.1	0.3	0.5	1	2	4	8
0.1		1		1			
0.1	1	4	2				
0.3	1	5	3				1
0.5		2	11	7	7	1	
1		1	10	10	5		
2		1	4	2		1	
4					1	1	

[Table/Fig-6]: MIC Distribution of POL vs. COL for all CR-KAPE isolates: (a) CRKP; (b) CRAB; (c) CRPA; and (d) CREC.
The heatmap illustrates the MIC distribution, where darker green shades indicate the highest number of isolates sharing the same MIC values or within one to two dilutions of another antibiotic. Lighter green shades represent fewer isolates with similar MIC values or within one to two dilutions higher or lower, while white boxes indicate the least number of isolates or those with MICs significantly above or below another antibiotic.

MIC (CTR/SBT) µg/mL	n (%) CRKP	n (%) CRAB	n (%) CREC
0.125/4	28 (28.3)	40 (45.4)	13 (27.1)
0.25/4	7 (7.1)	36 (41)	19 (39.5)
0.5/4	8 (8.1)	-	4 (8.3)
1/4	1 (1)	-	-
2/4	2 (2)	-	-
4/4	1 (1)	12 (13.6)	-
8/4	11 (11.1)	-	2 (4.2)
16/4	3 (3)	-	-
32/4	23 (23.2)	-	6 (12.5)
64/4	13 (13.1)	-	2 (4.2)
>64/4	2 (2)	-	2 (4.2)

[Table/Fig-7]: MIC distribution of CSE (Elores) at 4740 mg/L (~10 mM) EDTA.

CZA-AT synergy was further assessed in CRKP and CREC isolates with available molecular data. Among CRKP (n=57) isolates, 46 (80.7%) demonstrated synergistic activity. Among these synergy-positive isolates, the most frequent β-lactamase gene combination was *bla*CTX-M + *bla*NDM + *bla*OXA-48-like 12/46 (26%), followed by *bla*NDM + *bla*OXA-48-like 11/46 (24%) [Table/Fig-9].

Organism	CZA alone	CZA and AT result	n (%)
CRKP (n=137)	Susceptible	CZA (S) and AT (S)	17 (12.4)
		CZA (S) and AT (I)	0
		CZA (S) and AT (R)	28 (20.4)
	Total synergy (CZA-S)		**45 (32.8)**
	Resistant	CZA (R) and AT (S)	3 (2.2)
		CZA (R) and AT (I)	2 (1.5)
		CZA (R) and AT (R)	58 (42.3)
	Total synergy (CZA-R)		**63 (46.0)**
CREC (n=75)	Susceptible	CZA (S) and AT (S)	8 (10.7)
		CZA (S) and AT (I)	4 (5.3)
		CZA (S) and AT (R)	2 (2.7)
	Total synergy (CZA-S)		**14 (18.7)**
	Resistant	CZA (R) and AT (S)	6 (8.0)
		CZA (R) and AT (I)	4 (5.3)
		CZA (R) and AT (R)	21 (28.0)
	Total synergy (CZA-R)		**31 (41.3)**

[Table/Fig-8]: Synergy results of CZA–AT E-test disk diffusion assay among CRKP (n = 137) and CREC (n = 75), stratified by CZA susceptibility.
CRKP: Carbapenem-resistant Klebsiella pneumoniae; CREC: Carbapenem-resistant Escherichia coli

Among CREC isolates evaluated (n=17), 6 (35.3%) demonstrated CZA-AT synergy [Table/Fig-10]. Fosfomycin AD performed on *E. coli* urine isolates (n=42), showed 38 (90.5%) susceptibility.

Gene combination	CZA alone R			CZA alone S		
	AT (S)	AT (R)	Total (n)	AT (S)	AT (R)	Total (n)
*bla*NDM	0	3	3	0	0	0
*bla*OXA-48 LIKE	0	0	0	6	1	7
*bla*CTX-M+blaKPC	0	0	0	1	1	2
*bla*CTX-M+blaNDM	0	2	2	0	0	0
*bla*CTX-M+*bla*OXA-48 LIKE	0	0	0	4	1	5
*bla*CTX-M+*bla*NDM+*bla*OXA-48 LIKE	1	6	7	4	1	5
*bla*CTX-M+*bla*NDM+*bla*OXA-48 LIKE+*bla*VIM	0	1	1	0	0	0
*bla*KPC+*bla*IMP	0	0	0	0	1	1
*bla*KPC+*bla*VIM+*bla*IMP	0	0	0	1	0	1
*bla*NDM+*bla*OXA-48 LIKE	0	11	11	0	0	0
*bla*IMP+*bla*VIM	0	1	1	0	0	0
Total (n)			25			21

[Table/Fig-9]: Synergy of CZA and aztreonam against CRKP isolates (n = 46) in relation to β-lactamase gene combinations.

The table summarises CZA-AT synergy among CRKP isolates stratified by susceptibility to CZA alone and associated β-lactamase gene combinations. Isolates harboring metallo-β-lactamase genes, particularly blaNDM, either alone or in combination with other β-lactamases, showed a high frequency of CZA-AT synergy. In contrast, isolates possessing serine carbapenemases such as *bla*OXA-48-like alone were more frequently susceptible to CZA alone, with limited additional benefit observed with the addition of AT. Isolates with co-occurring resistance determinants, especially *bla*CTX-M+*blaNDM*+*bla*OXA-48-like, showed greater dependence on combination therapy

Gene combination	Synergy observed				No synergy observed			
	CZA alone R		CZA alone S		CZA alone R		CZA alone S	
	AT (S)	AT (R)	AT (S)	AT (R)	AT (S)	AT (R)	AT (S)	AT (R)
*bla*NDM	1	1	1	0	4	4	0	0
*bla*CTX-M + *bla*NDM	1	0	0	0	0	1	0	0
*bla*CTX-M+*bla*NDM+*bla*OXA-48 LIKE	1	0	0	0	-	-	-	-
*bla*NDM+*bla*OXA-48 Like	1	0	0	0	0	1	0	0
*bla*NDM & *bla*IMP	-	-	-	-	1	0	0	0

[Table/Fig-10]: Synergy in CREC (n = 17) isolates against CZA and AT in relation to single and co-occurring resistance genes.

All CREC isolates harbored blaNDM, either alone or in combination with other β-lactamase genes. A significant proportion (16/17; 94%) of isolates were resistant to CZA alone

DISCUSSION

The CR-KAPE are opportunistic nosocomial pathogens commonly encountered in ICUs, often associated with prolonged hospital stays and secondary infections involving central line catheters, urinary tract, respiratory tract, and wound sites. In the present study, the majority of CR-KAPE isolates were recovered from ICU settings, indicating their predominance in critical care environments. With increasing prevalence of CR-KAPE, identifying effective antimicrobials remains a critical priority especially when the new antimicrobial pipeline is limited [1-4].

Resistance to COL is increasingly reported particularly among CRKP and CRAB [17-19]. In the present study, 11.6% of CRKP isolates and 4.8% of CREC isolates were COL R. These findings are consistent with previous reports of COL R in CRKP ranging from 7% to 51.6% [20,21] and highlight the importance of identifying COL R among CR-KAPE isolates prior to initiating therapy to optimise patient outcomes.

Unlike COL (prodrug), POL has favourable pharmacokinetic and pharmacodynamic profiles, is not renally excreted, and is therefore preferred over COL at most sites of infection, except UTIs. While CLSI does not provide specific breakpoints for POL, COL breakpoints can be extrapolated for clinical use. In the current study, 93% of CRKP isolates exhibited intermediate susceptibility to POL, with 7% R, aligning with previous reports from China showing susceptibility rates of 91.8-94.9% and resistance of 5-8.2% [22]. Prior studies have reported that COL use may drive an increase in resistance among CR strains [8,23]. Polymyxins may also demonstrate heteroresistance and are therefore generally used in combination therapy to prevent potential in-vitro growth or emergence of resistance.

Fosfomycin is a reliable option for treating UTIs caused by *E. coli*, including CREC, which exhibited 90% S in the present study. A similar study reported 96.5% S [16], reinforcing the role of fosfomycin in both intravenous and oral targeted therapy for UTIs.

CZA-AT is a widely used regimen for MBL producing Enterobacterales, especially where MBLs and serine β-lactamases are co-expressed [24]. In the present study, synergy was observed in 79% of CRKP and 60% of CREC isolates, which was lower than previously reported rates of 95-100% among CR Enterobacterales [25,26].

Despite unclear literature on the in-vitro testing of CSE, clinical reports have shown good efficacy in treating patients with skin and soft-tissue, as well as bone and joint infections [27]. In the present study, CSE at a fixed concentration of SBT and EDTA exhibited 100% S against CRAB, with 86.4% of isolates showing lower MIC values between 0.25-0.125 μg/mL. Another study using EDTA at 10 mM with a CTR: SBT ratio of 2:1 reported MIC values ranging from 2-16 μg/mL against MBL-producing A. baumannii [28]. These findings suggest that CSE may be a promising treatment option for CRAB infections in combination with polymyxins; however, multi-centric studies are warranted to further validate these results.

This study provides a comprehensive assessment of AST for salvage antibiotics (COL, POL, CZA-AT, fosfomycin, and CSE) against MDR pathogens listed in the WHO Bacterial Priority Pathogens List (2024) [3] and Indian Priority Pathogens List (IPPL, 2021) [2], using CLSI-recommended reference methods (broth microdilution and agar dilution, as applicable) on over 300 clinical isolates. Overall, it contributes valuable data on the in-vitro activity of salvage agents and offers insights into their potential role in managing CR-KAPE infections in tertiary care settings. While data on the antimicrobial activity of CSE at a constant concentration of SBT and EDTA remain limited, the findings reported here provide preliminary evidence supporting further systematic evaluation. Future multicentric studies incorporating molecular characterisation and clinical outcome correlation would help validate these findings and strengthen their applicability in antimicrobial stewardship strategies.

Limitation(s)

A limitation of the study was the absence of genotypic analysis of β-lactamase-producing CR-KAPE isolates and the lack of evaluation of specific resistance mechanisms, including porin loss, efflux pump overexpression, ESBL/AmpC production combined with permeability defects, heteroresistance, biofilm formation, and the inoculum effect. These factors may contribute to discrepancies between in-vitro susceptibility results and clinical outcomes, potentially influencing therapeutic response despite apparent resistance. Addressing these aspects in future studies could provide a more comprehensive understanding of resistance patterns and underlying molecular determinants.

CONCLUSION(S)

The study highlights the limited and declining efficacy of salvage antibiotics against CR-KAPE infections. Overall, resistance to colistin was observed in some CRPA and CRAB isolates, while polymyxin B demonstrated complete in-vitro activity against CRPA and maintained high activity against other MDR pathogens. CZA-AT and fosfomycin demonstrated substantial activity, and CSE at a fixed concentration of SBT and EDTA showed promising results against CRAB, suggesting its potential as a therapeutic option. The use of CLSI-recommended reference AST methods, although labour-intensive and time-consuming, provided reliable susceptibility data to guide targeted therapy. These findings underscore the need for continuous surveillance of AMR trends and the development of rapid, standardised testing methods with high concordance to reference techniques. Judicious use of last-resort antibiotics remains essential to optimise patient outcomes and prevent further emergence of resistance.

REFERENCES

[1] Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, et al. Global burden of bacterial antimicrobial resistance 1990–2021: A systematic analysis with forecasts to 2050. The Lancet. 2024;404:1199-226. Available from: https://doi.org/10.1016/S0140-6736(24)01867-1.

[2] World Health Organization, Regional Office for South-East Asia. Indian priority pathogen list [Internet]. New Delhi: World Health Organization; 2021 [cited 2025 Aug 10]. Available from: https://cdn.who.int/media/docs/default-source/searo/india/antimicrobial-resistance/ippl_final_web.pdf.

[3] World Health Organization. WHO bacterial priority pathogens list 2024 [Internet]. Geneva: WHO; 2024 [cited 2025 Aug 10]. Available from: https://iris.who.int/server/api/core/bitstreams/1a41ef7e-dd24-4ce6-a9a6-1573562e7f37/content.

[4] Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, Pascual A. Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and carbapenemase-producing enterobacteriaceae. Clin Microbiol Rev. 2018;31. Available from: https://doi.org/10.1128/cmr.00079-17.

[5] Srivastava P, Bisht D, Kumar A, Tripathi A. Prevalence of carbapenem-resistant escherichia coli and klebsiella pneumoniae in rural Uttar Pradesh. Journal of Datta Meghe Institute of Medical Sciences University. 2022;17:584-88. Available from: https://doi.org/10.4103/jdmimsu.jdmimsu_76_22.

[6] Sharma K, Tak V, Nag VL, Bhatia PK, Kothari N. An observational study on carbapenem-resistant Enterobacterales (CRE) colonisation and subsequent risk of infection in an adult intensive care unit (ICU) at a tertiary care hospital in India. Infection Prevention in Practice. 2023;5:100312. Available from: https://doi.org/10.1016/j.infpip.2023.100312.

[7] Hsu L-Y, Apisarnthanarak A, Khan E, Suwantarat N, Ghafur A, Tambyah PA. Carbapenem-resistant *Acinetobacter baumannii* and enterobacteriaceae in south and southeast Asia. Clin Microbiol Rev. 2017;30:1-22. Available from: https://doi.org/10.1128/cmr.00042-16.

[8] Pace MC, Corrente A, Passavanti MB, Sansone P, Petrou S, Leone S, et al. Burden of severe infections due to carbapenem-resistant pathogens in intensive care unit. World J Clin Cases. 2023;11:2874-89. Available from: https://doi.org/10.12998/wjcc.v11.i13.2874.

[9] Tamma PD, Heil EL, Justo JA, Mathers AJ, Satlin MJ, Bonomo RA. Infectious Diseases Society of America 2024 guidance on the treatment of antimicrobial-resistant gram-negative infections. Clin Infect Dis. 2024. Available from: https://doi.org/10.1093/cid/ciae403.

[10] Clinical and Laboratory Standards Institute (CLSI) M100 Performance Standards for Antimicrobial Susceptibility Testing, 34thEdition, 2024 n.d.

[11] Rahmani S, Basu S, Simner PJ, Kambli P, Shetty A, Rodrigues C. Evaluation of the rapid lateral flow assay (LFA) for detection of five major carbapenemase enzyme families in genotypically characterised bacterial isolates. Indian J Med Res. 2025;161:699-705. Available from: https://doi.org/10.25259/IJMR_1976_2024.

[12] Weinstein MP, Patel JB. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: M07-A11. 11. edition. Wayne, PA: Committee for Clinical Laboratory Standards; 2018.

[13] Chaudhary M. Molecular characterization and antimicrobial susceptibility study of Acinetobacter baumannii clinical isolates from middle east, African and Indian Patients. J Proteomics Bioinform. 2012;05. Available from: https://doi.org/10.4172/jpb.1000248.

[14] Chaudhary M, Sudaroli M, Kumar S, Krishnaraju V. Catering ESBL resistance challenge through strategic combination of ceftriaxone, sulbactam and ethylenediaminetetraacetic acid. Int J Drug Development and Res. 2012;4:72-81.

[15] Rawson TM, Brzeska-Trafny I, Maxfield R, Almeida M, Gilchrist M, Gonzalo X, et al. A practical laboratory method to determine ceftazidime-avibactam-aztreonam synergy in patients with New Delhi metallo-beta-lactamase (NDM)–producing Enterobacterales infection. J Glob Antimicrob Resist. 2022;29:558-62. Available from: https://doi.org/10.1016/j.jgar.2022.01.025.

[16] Pereira JV, Bari AK, Kokare R, Poojary A. Comparison of in-vitro fosfomycin susceptibility testing methods with agar dilution for carbapenem resistant *Klebsiella pneumoniae* and *Escherichia coli*. Indian J Med Microbiol. 2023;42:39-45. Available from: https://doi.org/10.1016/j.ijmmb.2023.01.005.

[17] Satlin MJ. The search for a practical method for colistin susceptibility testing: Have we found it by going back to the future? J Clin Microbiol. 2019;57:e01608-18. Available from: https://doi.org/10.1128/JCM.01608-18.

[18] Sacco F, Visca P, Runci F, Antonelli G, Raponi G. Susceptibility testing of colistin for *Acinetobacter baumannii*: How far are we from the truth? Antibiotics. 2021;10:48. Available from: https://doi.org/10.3390/antibiotics10010048.

[19] Bernhardt JS, Tellis RC, Motagi A, Asif PP. Colistin and Polymyxin B sensitivity by broth microdilution among carbapenem-resistant clinical isolates of Gram-negative bacilli. Biomedicine. 2024;43:1789-93. Available from: https://doi.org/10.51248/.v43i6.3164.

[20] Basavaraju M, Rao MR, Shankaregowda R, Mahale R, Shivappa SG, Chitharagi VB. Comparative evaluation of broth microdilution, E-strip and Vitek-2 for colistin susceptibility among carbapenem resistant *Acinetobacter* spp and *Klebsiella* spp. IJMR. 2024;11:243-47. Available from: https://doi.org/10.18231/j.ijmr.2024.043.

[21] Koçer I, Zer Y, Buyuktas A. The detection of colistin resistance in carbapenem resistant *Klebsiella pneumoniae* isolates. Int J Adv Med. 2021;8:357. Available from: https://doi.org/10.18203/2349-3933.ijam20210592.

[22] Jia X, Yin Z, Zhang W, Du S. The efficacy and influencing factors of polymyxin B in high-level Carbapenem-Resistant *Klebsiella pneumoniae* infections. Infect Drug Resist. 2023;16:4177-87. Available from: https://doi.org/10.2147/IDR.S409090.

[23] Narimisa N, Goodarzi F, Bavari S. Prevalence of colistin resistance of Klebsiella pneumoniae isolates in Iran: A systematic review and meta-analysis. Ann Clin Microbiol Antimicrob. 2022;21. Available from: https://doi.org/10.1186/s12941-022-00520-8.

[24] 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. Available from: https://doi.org/10.1186/s12941-023-00573-3.

[25] Ruan X, Gong Z, Zeng M, Zhong Z, Chen Y, Wei F, et al. Aztreonam acts as a synergist for ceftazidime/avibactam against Carbapenem-Resistant Enterobacteriaceae (CRE) of various carbapenemase phenotypes in southwestern China. IDR. 2025;18:2243-53. Available from: https://doi.org/10.2147/idr.s523498.

[26] Wu Y, Yu W, Chu X, Zhang J, Jia P, Liu X, et al. Effect of ceftazidime-avibactam combined with different antimicrobials against carbapenem-resistant *Klebsiella pneumoniae*. Microbiol Spectr. 2024;12. Available from: https://doi.org/10.1128/spectrum.00107-24.

[27] Chaudhary M, Mir MA, Ayub SG. Safety and efficacy of a novel drug elores (ceftriaxone + sulbactam + disodium edetate) in the management of multi-drug resistant bacterial infections in tertiary care centers: A post-marketing surveillance study. The Brazilian Journal of Infectious Diseases. 2017;21:408-17. Available from: https://doi.org/10.1016/j.bjid.2017.02.007.

[28] Chaudhary M, Payasi A. Synergy of a novel antibiotic adjuvant entity against multidrug-resistant Enterobacteriaceae. Am J Infect Dis [Internet]. 2013 [cited 2025 Aug 10];9(3):94-103. Available from: https://doi.org/10.3844/ajidsp.2013.94.103.

PARTICULARS OF CONTRIBUTORS:

1. Research Fellow and PhD Scholar, Department of Microbiology, Breach Candy Medical Research Centre, Mumbai, Maharashtra, India.
2. Laboratory Director, Department of Pathology and Microbiology, Breach Candy Hospital Trust and Breach Candy Medical Research Centre, Mumbai, Maharashtra, India.
3. Doctoral Researcher, Department of Medical Microbiology and Infection Prevention, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Prerna S Salian,
60 A, Bhulabhai Desai Marg, Breach Candy, Cumballa Hill, Mumbai-400026,
Maharashtra, India.
E-mail: prernassalian@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 28, 2025
- Manual Googling: Apr 25, 2026
- iThenticate Software: Apr 27, 2026 (8%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 08, 2025**
Date of Peer Review: **Jan 05, 2026**
Date of Acceptance: **Apr 29, 2026**
Date of Publishing: **Aug 01, 2026**