

## Original Research Article

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# A case-control study on clinical outcome and risk factors associated with carbapenem resistant isolates from critical care unit at a tertiary care hospital in Gujarat

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## ABSTRACT

**Background:** The increasing prevalence of carbapenemase-producing organisms (CPO) is concerning because of the rapid dissemination of carbapenemase genes. Main objectives of the work were to study the prevalence of CPO and their genes using the Xpert® Carba-R assay in bacterial isolates from critical care settings, as well as to study patient-specific risk factors and comorbidities associated with carbapenem-susceptible organisms (CSO) and carbapenem-resistant organisms (CRO).

**Methods:** A prospective, observational, case-control study was conducted from January 2023 to March 2024. Bacterial identification & susceptibility testing was done using VITEK® 2 Compact and Kirby-Bauer disc diffusion method. Study consisted of 100 patients (50 carbapenem-resistant cases and 50 carbapenem-susceptible controls). CRO were further studied for carbapenemase production using the Xpert® Carba-R assay test to detect the *bla*<sub>KPC</sub> (KPC), *bla*<sub>NDM</sub> (NDM), *bla*<sub>VIM</sub> (VIM), *bla*<sub>OXA-48</sub> (OXA-48), and *bla*<sub>IMP</sub> (IMP) genes.

**Results:** Carbapenem Resistant *Acinetobacter baumannii* (CRAB) [40.35%] was the most common isolate, followed by Carbapenem Resistant *Pseudomonas aeruginosa* (CRPA) [23.95%], Carbapenem Resistant *Klebsiella pneumoniae* (CRKP) [21.52%], and Carbapenem Resistant *Escherichia coli* (CREC) [15.27%]. CRKP showed the highest carbapenemase production (42%). NDM carbapenemases were most common (54%), followed by NDM + OXA-48 co-production (30%). No genes were detected in 12% of isolates. IMP, VIM, and KPC genes were not detected. Carbapenem resistance was significantly associated with male sex, lengthy hospital stays, in-situ Foley's catheter, and multiple co-morbidities. Mortality was 14% in cases and 4% in controls (OR, 3.9; 95% CI, 0.76 to 19.83).

**Conclusions:** In conclusion, CRO infections were associated with multiple risk factors, longer hospital stays and higher mortality compared to CSO infections.

**Keywords:** Carbapenem resistant Enterobacteriales, Carbapenemase producing organisms, New Delhi metallo-β-lactamases

## INTRODUCTION

Carbapenems are critical antibiotics for treating systemic infections caused by multidrug-resistant gram-negative bacteria. Recently, the rise in carbapenem resistance has become a public health issue. WHO global priority list categorizes Carbapenem-Resistant Enterobacteriaceae

(CRE) and Carbapenem-Resistant *Acinetobacter baumannii* (CRAB) as critical, and Carbapenem-Resistant *Pseudomonas aeruginosa* (CRPA) in the high-priority group.<sup>1</sup> Antimicrobial resistance arises from different mechanisms with β-lactamases being a common reason. Carbapenemases, a type of β-lactamases has three types; A, B, and D.<sup>2</sup> Class A includes KPCs, widely distributed

among Enterobacteriaceae.<sup>3</sup> Class B includes metallo-β-lactamases like IMP, VIM, and NDM.<sup>2,3</sup> Class D OXA β-lactamases, notably OXA-48 variants, are found in *Acinetobacter* species and Enterobacteriaceae.<sup>4,5</sup> Identifying the type of carbapenemase produced is important as it has treatment implications and guides infection control measures.

Both phenotypic and molecular assays are used for detection of carbapenemase-producing organisms (CPO). Phenotypic methods include growth-based assays (e.g., Modified Hodge Test), hydrolysis methods (e.g., Carba NP, MALDI-TOF MS), and lateral flow immunoassays but have limited sensitivity and specificity with inability to identify specific carbapenemases. Molecular methods, like PCR techniques (simplex, multiplex, real-time), hybridization and DNA microarrays are considered the gold standard. Whole Genome Sequencing (WGS) is also used for comprehensive detection but they have limited availability.

Xpert® Carba-R assay, a real-time PCR assay detects *bla*<sub>KPC</sub> (KPC), *bla*<sub>NDM</sub> (NDM), *bla*<sub>VIM</sub> (VIM), *bla*<sub>OXA-48</sub> (OXA-48), and *bla*<sub>IMP</sub> (IMP) genes with high sensitivity (96.6%) and specificity (98.6%), with results in 32-48 minutes and thus sounds promising for clinicians.<sup>6</sup> This study conducted at a tertiary care teaching hospital in Gujarat, aims to identify the types of carbapenemase genes in resistant bacterial isolates using the Xpert® Carba-R assay and evaluate changes in antimicrobial treatment with its impact on patient outcomes, addressing gaps in current research on treatment modification post-carbapenemase detection. Our research hypothesis was that the detection of carbapenemase gene in carbapenem resistance isolates will help in improving patient's outcome through choice of right antimicrobial therapy.

## METHODS

This prospective, observational case-control study was conducted from January 1, 2023 to March 31, 2024 following approval from Institutional Ethics Committee in the Microbiology section of NABL-accredited Central Diagnostic Laboratory (CDL) at NABH accredited Shree Krishna Hospital, Karamsad, Gujarat, a 1000-bedded tertiary care facility with 102-bed ICU/CCU.

Any isolate of Carbapenem-resistant *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* from any clinical specimen, with an Xpert® Carba-R assay request was included as a CRO (Case) while any of these isolates if Carbapenem-susceptible, isolated from the patients admitted to the same type of hospital location as cases was included as CSO (Control). Repeat isolates from the same specimen (patient) were excluded.

Definitions given by CDC were used for carbapenem resistant Enterobacteriaceae (CRE), carbapenem resistant *P. aeruginosa* (CRPA), carbapenem resistant *A. baumanii* (CRAB) and Carbapenemase Producing Carbapenem-

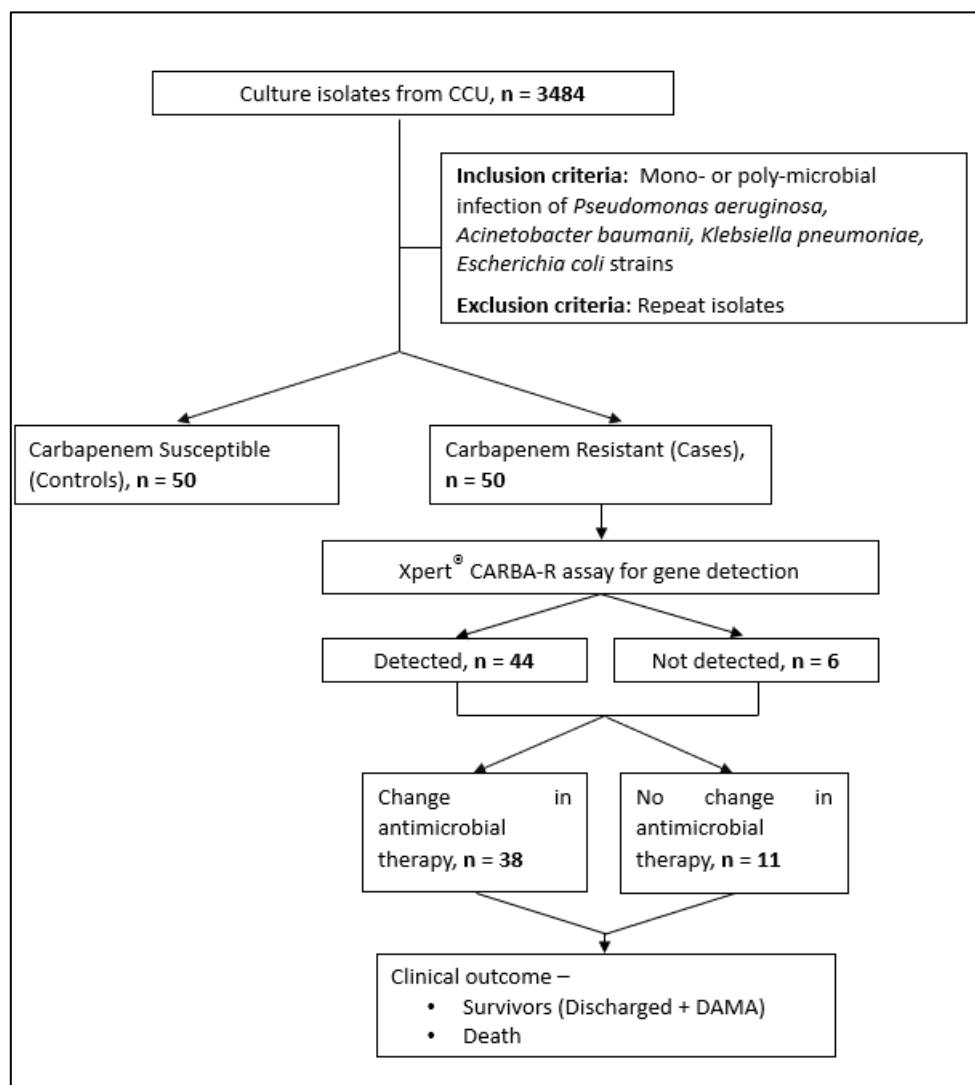
Resistant Enterobacteriaceae (CP-CRE).<sup>7</sup> Cases included carbapenem-resistant strains of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*, while controls included susceptible isolates. Each CRO case was matched with a CSO control (1:1) from the same type of CCU. A total of 50 isolates in each case/control were studied. Demographic (Age, Sex, Date of admission, length of stay) and clinical details including various co-morbidities, presence of invasive devises, prescribed antimicrobial therapy and quick SOFA score at admission (day 0) of the patients were obtained from institutional Hospital Information System or direct visit to patient and consultant.

All microbiological procedures including sample collection, transportation and processing were performed using accepted, standard microbiological protocols. VITEK 2 automated system (bioMérieux) was used for Antimicrobial Susceptibility Tests (AST) and minimum inhibitory concentration (MIC) using the interpretative criteria in the Clinical Laboratory Standards Institute (CLSI) M100-Ed33 document. Carbapenemase production in the carbapenem resistant isolates was performed using the Xpert® Carba-R assay test. The primers and probes in the Xpert Carba-R assay detect *bla*<sub>KPC</sub> (KPC), *bla*<sub>NDM</sub> (NDM), *bla*<sub>VIM</sub> (VIM), *bla*<sub>OXA-48</sub> (OXA-48), and *bla*<sub>IMP</sub> (IMP) gene sequences. The change in antimicrobial therapy after carbapenemase detection was noted.

Data management and analysis used IBM SPSS 20.0 software. Age was categorized into four groups: 0–17, 18–44, 45–64, and ≥65 years. Frequency distributions for age and gender were calculated for both cases and controls. Length of stay (LOS) was measured from admission to discharge, and the mean LOS was determined. Co-morbidities were grouped into four categories (Patients with 0 co-morbidities, 1 co-morbidity, 2 co-morbidities and ≥ 3 co-morbidities). Chi-square or Fisher's exact tests compared proportions. Univariate comparisons assessed the influence of variables, with significant covariates selected for multivariate analysis using stepwise forward logistic regression. Odds ratios (OR) for factors associated with carbapenem-resistant organisms were calculated, with statistical significance set at  $p \leq 0.05$ .

## RESULTS

In our 16-month observational study, we compared risk factors and clinical outcomes between patients with carbapenem-resistant and carbapenem-susceptible infections caused by *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *A. baumannii* in CCUs. Out of 3,484 samples, 582 were positive for these bacteria, with 288 being carbapenem-resistant (49.48% prevalence). Amongst these 288 carbapenem resistant isolates, 50 isolates were further tested for carbapenemase detection by Xpert® Carba-R assay which formed the 'case' group of our study. Similarly, among the 294 carbapenem susceptible isolates, 50 were taken as matched 'control' group. The outline of the study was described in Figure 1.



**Figure 1: Study outline.**

**Table 1: Univariate analysis of Cases v/s. Control groups.**

Characteristics	Cases, N (%)	Controls, N (%)	P value
<b>Sex</b>			
Male	44 (88)	28 (56)	
Female	6 (12)	22 (44)	0.00
<b>Age group in years</b>			
0-17	2 (4)	5 (10)	
18-44	16 (32)	7 (14)	
45-64	13 (26)	16 (32)	0.149
≥65	19 (38)	22 (44)	
<b>Invasive devices</b>			
CVC	17 (34)	0 (0)	0.00
Foley's catheter	36 (72)	26 (52)	0.039
ET/TT	30 (60)	28 (50)	0.685
Dialysis DLC	0 (0)	1 (2)	0.315
<b>Total invasive devices</b>			
0	14 (28)	14 (28)	
1	5 (10)	18 (36)	0.00

Continued.

Characteristics	Cases, N (%)	Controls, N (%)	P value
2	15 (30)	17 (34)	
3	16 (32)	1 (2)	
<b>Total co-morbidities</b>			
0	13 (26)	16 (32)	
1	6 (12)	13 (26)	
2	18 (36)	8 (16)	0.08
≥3	12 (24)	13 (26)	
<b>Length of hospital stay-Mean days (SD)</b>	43.16 ( $\pm 2.79$ )	17.44 ( $\pm 1.05$ )	0.00
<b>qSOFA at admission-Mean (SD)</b>	0.82 ( $\pm 0.05$ )	1.08 ( $\pm 0.18$ )	0.126

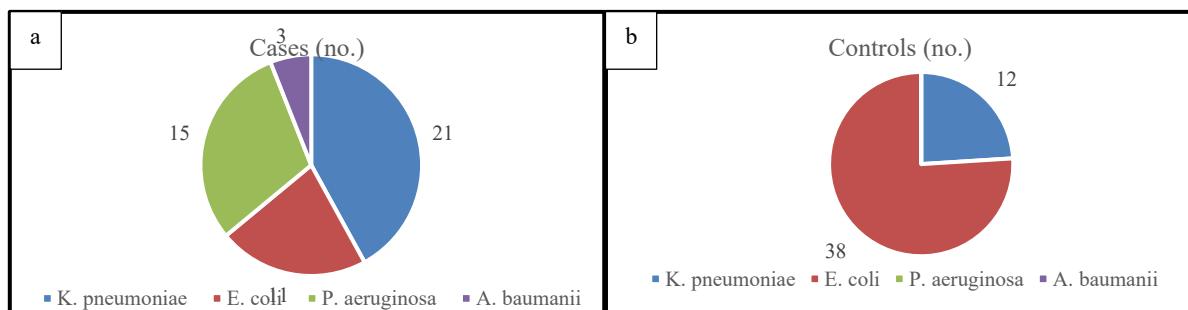


Figure 2 (a and b): Organisms isolated in cases and control groups.

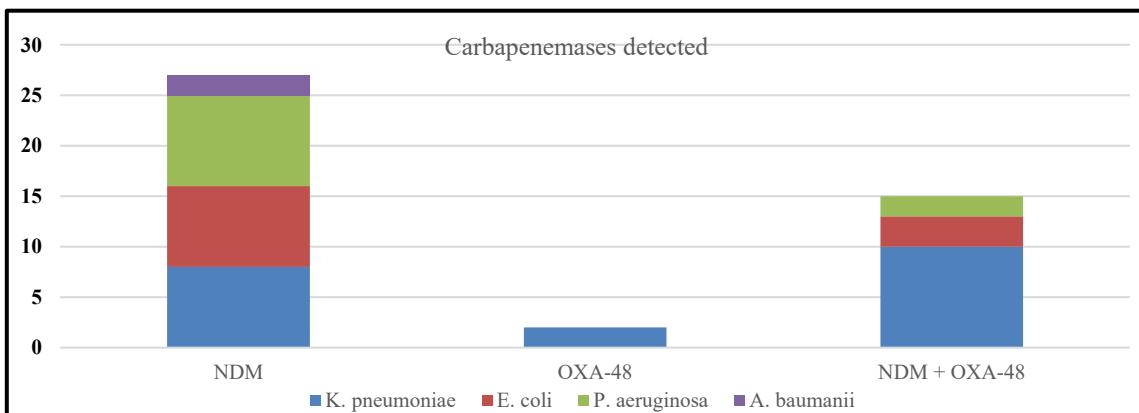


Figure 3: Carbapenemases detected in different CRO in the study.

Univariate analysis of demographic and clinical data of the 'cases' and 'controls' group are summarized in Table 1.

The organisms isolated in both the groups are depicted in the Figure 2.

The type of carbapenemases detected in cases are shown in the Figure 3.

Table 2 shows the clinical outcome of both the groups till they were discharged.

Table 3 shows the change in antimicrobial therapy after detection of carbapenemase in cases alone and their clinical outcome.

Table 2: Clinical outcome among cases versus control groups.

Clinical outcome	Cases, N (%)	Control, N (%)	Total, N (%)
<b>Survivors (Discharged+DAMA)</b>	43 (47.3)	48 (52.7)	91 (100)
<b>Deaths</b>	7 (77.8)	2 (22.2)	9 (100)
<b>Total</b>	50	50	100

**Table 3: Effect of change of antimicrobial therapy on clinical outcome.**

Change in antimicrobial therapy	Final outcome, N (%)		Total, N (%)	P value
	Survivors (Discharged + DAMA)	Deaths		
Yes	33 (86.84)	5 (13.16)	38 (100)	
No	9 (81.82)	2 (18.18)	11 (100)	0.69
No record available	1 (100)	0 (0)	1 (100)	

**Table 4: Adjusted Odd's ratio (OR) from final multivariate Logistic Regression Model.**

Variables	P value	Adjusted odds ratio	95% C.I.	
			Lower	Upper
Sex, Male	0.016	7.20	1.45	35.71
Length of hospital stay	0.025	1.03	1.004	1.059
≥ 3 invasive devices	0.037	24.24	1.21	1.059
2 co-morbidities	0.020	14.00	1.52	128.83

The Logistic regression model from final multivariate analysis is shown in the Table 4.

## DISCUSSION

### Carbapenem resistant isolates from critical care unit

Carbapenem resistance in ICUs and CCUs is a growing concern due to its impact on patient outcomes and infection control. In our study, total 288 CRE (CRKP and CREC only), CRPA and CRAB were isolated from critical care unit. Majority among these isolates were that of CRAB (40.35%), followed by CRPA (23.95%), CRKP

(21.52%) and CREC (15.27%). As per the annual report of Antimicrobial Resistance Research and Surveillance Network (AMRSN) 2022 [a division of Indian Council of Medical Research (ICMR)], *Acinetobacter baumanii* (23.6%) was the most commonly isolated organism followed by *K. pneumoniae* (21.5%), *E. coli* (12.9%) and *P. aeruginosa* (12.3%) from intensive care units across India.<sup>8</sup>

The overall prevalence of CRO was 49.48% which was still higher compared to other studies in India from 2012, as shown in Table 5.

**Table 5: Prevalence of carbapenem resistant organisms in India in last decade.**

Studies	Place of study	Year of study	Overall prevalence of CRO, %
Ralte et al <sup>9</sup>	North-east India	2018-19	11.3
Prabhala et al <sup>10</sup>	Western India	2022	26.5
Verma et al <sup>11</sup>	Eastern India	2022	29.5
Mate et al <sup>12</sup>	North-east India	2012-14	30
Parijat et al <sup>13</sup>	Western India	2016-17	31.14
Present study	Western India	2023-24	49.48

**Table 6: Prevalence of OXA-48 and OXA-like genes in India since 2010.**

Organism	Study type, and year of study	Percent of OXA producing isolates	Remarks	Reference
Enterobacteriaceae	SMART study, 2010	4.5	Only 3 isolates with OXA-48 were reported from India	21
Enterobacteriaceae	Single center, 2012	1.8	Reported in <i>K. pneumoniae</i> and <i>C. freundii</i>	22
<i>E. coli</i>	Single center, 2013	55.55	Only <i>E. coli</i> included	23
Enterobacteriaceae	Single center, 2016	24.7	<i>E. coli</i> and <i>K. pneumoniae</i> included	24

### Genotype profile among carbapenem resistant isolates

Among 50 isolates tested by Xpert® Carba-R assay, 88% harbored carbapenemase genes, with NDM being the most common (54%). Prabhala et al in 2022 reported a 33%

prevalence of NDM.<sup>10</sup> Other studies showed NDM prevalence of 53.4% in North India (2008-12), 35.7% in China (2016-18), and 50% in Egypt (2019).<sup>14-16</sup> In our study, 30% of isolates had both NDM and OXA-48, a higher co-producer rate compared to Anandan et al in

2013, who found 12.5%.<sup>17</sup> The study also found no KPC, IMP, or VIM, which matches our results.

KPC was absent in our study, aligning with findings from Garg et al in 2014-2016 in western India, which reported a 20% prevalence of co-producers but no KPC.<sup>18</sup> Unlike their study, our study found no VIM, whereas they reported 18.5% VIM. Similarly, KPC was absent in a Mexican study in 2022, which found 47% NDM and 27% IMP.<sup>19</sup> OXA-48 and similar enzymes are now predominant in India, seen in Enterobacteriaceae (Table 6). OXA-48 was present in 4% of *K. pneumoniae* isolates but not in *E. coli*. This absence in non-fermenters matches findings from studies in China and Iran.<sup>15,20</sup>

Out of 50 CRPA isolates, carbapenemase genes were detected in 15 and were absent in 4 isolates. Majority showed NDM (60%), and 13% had both NDM and OXA-48. Verma et al in 2018 found 62.74% of CRPA isolates with at least one carbapenemase gene, with VIM (46.87%) and NDM-1 (45.31%) being most common.<sup>25</sup> Whereas, VIM was not detected in our study. Like our results, their study also found no KPC, IMP, or OXA-48. Variations in molecular epidemiology among CR isolates are likely due to varying empirical antibiotic use across regions and study periods.

#### ***Risk factors associated with carbapenem resistance***

Assessing predictors of carbapenem resistance through univariate analysis in our study revealed associations between carbapenem-resistant infection and factors like male sex, length of hospital stay, central venous catheter placement, Foley's catheter placement, and presence of higher number of co-morbidities. In the multivariable analysis, male sex (OR, 7.20; 95% CI, 1.45 to 35.71), Length of hospital stay (OR, 1.03; 95% CI, 1.004 to 1.059), presence of  $\geq 3$  invasive devices (OR 24.24; 95% CI, 1.21 to 484.32), and presence of 2 or more comorbidities (OR 14; 95% CI, 1.52 to 128.83) emerged as independently linked to carbapenem resistance.

A systematic review which included 92 studies conducted between 2007 to 2018, identified previous antibiotic use (91.1%; 72/79 studies); mechanical ventilation (66.7%; 36/54); dialysis (61.1%; 11/18); catheter (58.0%; 40/69); length of stay in hospital (54.5%; 30/55); comorbidities (52.7%; 39/74); and intubation (51.4%; 18/35) as the most frequently reported risk factors with significant association with CR infection.<sup>26</sup>

An observational study conducted at a tertiary care hospital in western India reported that the longer a patient was in contact with any medical device, the greater was their likelihood of contracting a CRE infection.<sup>27</sup> Another study in North India (Gupta et al in 2020), revealed that the presence of Foley catheter (OR-6.21; 95% CI- 1.61-23.98;  $p=0.008$ ) were associated with CRKP BSI.<sup>28</sup>

A south-east Asian study by Ling et al from 2011 to 2013 revealed presence of central line device (OR: 3.117; 95 % CI: 1.167-8.330) as significant independent predictor.<sup>29</sup> A research done in USA (2008) by Patel et al, and other two researches done in Greece in 2007-08 by Souli et al, as well as 2003-07 by Falagas et al has identified comparable risk factors for carbapenem-resistant infections, including associations with prolonged hospitalization, ICU admission, central venous catheter usage, recent solid-organ or stem-cell transplantation, and utilization of mechanical ventilation.<sup>30-32</sup>

#### ***Clinical outcome***

CRE resistance is contingent on various factors including the patient's health status, recent transplant history, risk of co-infection, and the utilization of multiple antibiotics.<sup>33</sup> A study conducted in North India between 2011 to 2015 by Kaur et al showed a high in-hospital mortality rate of 69.3% among CRKP BSI.<sup>34</sup> A study at a tertiary oncology centre in India by Hari et al from 2017 to 2018 also showed a high mortality (69%) among patients suffering from acute leukaemia who have CRO infections.<sup>35</sup> Our study showcased an overall mortality rate of 14%, which is fortunately less compared with the 32% mortality reported in another analogous multicentre study from the USA focusing on the utilization of ceftazidime-avibactam in CRE cases by King et al.<sup>36</sup>

#### ***Effect of change of antimicrobial therapy***

Research shows a strong link between carbapenem use and increased carbapenem resistance in ICUs.<sup>37</sup> Culture-guided therapy, which tailors antibiotic treatment based on culture and sensitivity results, has been effective. Reyes et al in 2018-19 found that such an approach reduced mortality from 29% to 15%.<sup>38</sup> In Norway (2005), Berlid D. and colleagues adjusted antibiotic use in 88% of cases, narrowing therapy in 80% and reducing antibiotic use by 22%, with a 23% lower cost for adjusted therapy, though it did not impact mortality.<sup>39</sup> Altaf et al in 2018 in Pakistan also adjusted antibiotics based on culture results but found no effect on clinical outcomes.<sup>40</sup>

Limitations include single-centre data, lack of access to pre-admission records, non-hospital antibiotic exposure data, or other factors not documented in hospital charts. Future research could involve diverse populations and adding other Enterobacteriales members to evaluate CRE extensively.

#### ***CONCLUSION***

In conclusion, this study found that male sex and the use of invasive devices like central venous catheters and Foley's catheters are significant risk factors for CRO infections. CRO infections were associated with higher morbidity and longer hospital stays compared to CSO infections. However, changes in antimicrobial therapy did not significantly impact clinical outcomes ( $p=0.69$ ),

rejecting our hypothesis. The study suggests that patient medical history could help identify those at risk for CP-CRE.

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