Annals of Clinical Microbiology and Antimicrobials

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Clinical and Microbiological characteristics of patients with ceftazidime/avibactam-resistant *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* strains

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Abstract

Background Ceftazidime/avibactam (CZA)-resistant *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* has emerged, typically due to mutations in the bla_{KPC} gene. This study aimed to investigate the clinical and microbiological characteristics of patients with CZA-resistant KPC-producing *K. pneumoniae*, with a focus on comparing strains with KPC variants to those with wild-type KPC.

Methods Unique adult patients with CZA-resistant KPC-producing *K. pneumoniae* were identified at Taipei Veterans General Hospital between February 2019 and June 2024. Clinical characteristics and outcomes were recorded, and KPC variants were detected using polymerase chain reaction followed by Sanger sequencing.

Results A total of 60 cases of CZA-resistant KPC-producing *K. pneumoniae* were included. The 14-day and in-hospital mortality rates were 20% and 41.7%, respectively. Thirty-six strains (60%) harbored KPC variants, with 22 different types identified. KPC-33 ($n=12$) was the most common variant. Previous isolation of carbapenem-resistant *K. pneumoniae* and prior exposure to CZA were more common in the KPC variant group than in the wild-type KPC group. Strains producing KPC variants showed a higher proportion of CZA minimum inhibitory concentration (MIC) ≥ 64 μg/mL (80.6% vs. 4.2%, $p<0.001$) and restored meropenem susceptibility (MIC ≤ 4 μg/mL) (72.2% vs. 0%, $p<0.001$) compared to those producing wild-type KPC. Additionally, the 14-day mortality rate was lower in patients infected with KPC variant strains compared to those with wild-type KPC strains (11.5% vs. 36.4%, $p=0.041$).

Conclusion CZA-resistant KPC-producing *K. pneumoniae* is associated with high mortality. Strains producing KPC variants are more likely to exhibit restored meropenem susceptibility and higher levels of CZA resistance.

Keywords Ceftazidime/avibactam resistance, Klebsiella pneumoniae carbapenemase, KPC variants, Meropenem

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Introduction

Carbapenem-resistant Enterobacterales (CRE) infections have become a global public health challenge, primarily due to the spread of plasmids carrying carbapenemases genes [1]. Among CRE, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is the most prevalent species, with *K. pneumoniae* carbapenemase (KPC) being the predominant carbapenemase [2], followed by oxacillinase (OXA)-48-like β-lactamases and metallo-β-lactamases (MBL) worldwide.

Ceftazidime/avibactam (CZA), a novel β-lactam/β-lactamase inhibitor, is effective against KPC- and OXA-48-like-producing CRE but not MBL-producing strains [3]. Approved by the U.S. Food and Drug Administration in 2015, CZA is used to treat complicated urinary tract infections and complicated intra-abdominal infections [4]. In Europe, CZA was also approved by European Medicines Agency in 2016 for hospital-acquired pneumonia/ventilator-associated pneumonia, bacteremia associated with any of the above infections and infections caused by aerobic Gram-negative bacteria when other treatments might not work [5]. The Infectious Diseases Society of America guidance recommends meropenem/vaborbactam, CZA, imipenem/cilastatin/relebactam as preferred treatment options for KPC-producing Enterobacterales infections, with cefiderocol as an alternative option. Tigecycline or eravacycline are alternative options for the treatment of KPC-producing infections not involving the bloodstream or urinary tract [3]. However, resistance to CZA has emerged in clinical settings, with rates around 10% reported in several studies [6–8]. The resistance mechanism is often linked to mutations in the bla_{KPC} gene [2], though other mechanisms, such as alterations in outer membrane porins and overexpression of bla_{KPC} have also been implicated [9]. Strains with KPC variants, such as KPC-31 and KPC-33, which contain the D179Y substitution in the Ω-loop, exhibit resistance to CZA while showing restored susceptibility to meropenem [10, 11]. Clinical studies on patients infected with CRKP strains producing KPC variants are limited [2, 12, 13]. The in-hospital mortality rate of CZA-resistant, KPC-producing *K. pneumoniae* bacteremia was 22% in one study [12]. Another study reported an all-cause mortality rate of 33.9% among patients infected with CZA-resistant, KPC-producing CRKP [13], highlighting its emergence as a new public health challenge [2].

CZA was introduced in Taiwan in 2019 and is recommended as the preferred agent for treating CRE infections in national guidelines [14]. This study aimed to investigate the clinical and microbiological characteristics of patients with CZA-resistant, KPC-producing CRKP, with a specific focus on comparing the features between wild-type KPC (KPC-2 or KPC-3) and KPC variants.

Methods

Study design and setting

This single-center, observational, retrospective study was conducted at Taipei Veterans General Hospital, a tertiary medical center in northern Taiwan, focusing on hospitalized patients with ceftazidime/avibactam (CZA)-resistant, KPC-producing carbapenem-resistant *K. pneumoniae* (CRKP) isolated between February 2019 and June 2024. The inclusion criteria were adult patients (≥ 20 years old) who were either infected or colonized with a CZA-resistant, KPC-producing CRKP strain for the first time during their index hospitalization. Data were collected through chart reviews and included the following: demographics, pre-existing medical conditions, source of infection, disease severity, microbiological data, antibiotic regimens, and outcomes. Disease severity was assessed using the Sequential Organ Failure Assessment (SOFA) score and the Acute Physiology and Chronic Health Evaluation II (APACHE II) score. Clinical outcomes included 14-day mortality, 28-day microbiological eradication, relapse within 28 days after treatment, secondary infection within 28 days after treatment, and all-cause mortality. The study protocol was approved by the Institutional Review Board of Taipei Veterans General Hospital and informed consent was waived.

Definitions

Patients without clinical symptoms or signs attributable to the index CRKP were considered colonized [15]. Septic shock was defined as persistent hypotension requiring vasopressors to maintain a mean arterial pressure (MAP) ≥ 65 mmHg, with a serum lactate level > 2 mmol/L (18 mg/dL) [16]. Appropriate therapy was defined as receiving an active drug against the isolate for at least 48 h, initiated within the first 5 days of infection [17]. Clinical success was defined as survival and resolution of infection signs and symptoms at 28 days following the onset of infection [8]. Microbiological eradication was defined as the absence of CRKP from all subsequent cultures within 28 days after treatment initiation [13]. Relapse was defined as the onset of second microbiologically documented CRKP infection in the 28 days after the end of treatment in a patient who had previously achieved clinical cure and microbiological response [13]. Secondary infection was defined as an infection caused by a microorganism other than CRKP within 28 days after the start of treatment [13]. Immunocompromised status included neutropenia, acquired immunodeficiency syndrome (AIDS), or receiving corticosteroids or other immunosuppressant therapy for more than 4 weeks.

Bacterial identification and antimicrobial susceptibility testing

CRKP strains were isolated from clinical samples, and bacterial identification was determined using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) (bioMérieux SA, Marcy l'Etoile, France). Antimicrobial susceptibility was determined using the Vitek2 System (bioMérieux, Marcy l'Etoile, France). The minimum inhibitory concentration (MIC) of CZA was determined using either the broth microdilution method or E-test strips. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, with a CZA susceptibility breakpoint of 8/4 μg/mL [18].

bla$_{KPC}$ gene detection and KPC variant identification

DNA was extracted from CRKP isolates for polymerase chain reaction (PCR) amplification. Genes coding for carbapenemases, including KPC, NDM, IMP, VIM, and OXA-48, were detected by PCR as previously described [19]. All CZA-resistant, *bla*$_{KPC}$-harboring isolates underwent gene sequencing to identify KPC variants. Multilocus sequence typing (MLST) was performed through PCR amplification and Sanger sequencing, as previously described [20].

Statistical analysis

Categorical variables were expressed as numbers and percentages, while continuous variables were expressed as medians and interquartile ranges (IQR). Differences between the KPC variant and wild-type KPC groups were compared using the Chi-square and Fisher's exact tests for categorical data, and the Student's t test and Mann-Whitney U test for continuous data. Univariate and multivariate logistic regression analyses were performed to identify independent variables associated with 14-day mortality. Variables with a p-value < 0.1 in the univariate analysis were included in the multivariate models. A backward selection method was used for multivariate logistic regressions. The Kaplan-Meier survival analysis was performed for the comparison of 14-day mortality between wild-type KPC group and KPC variants group. All p-value analyses were two-sided, with a p-value < 0.05 considered statistically significant. All statistical analyses were performed using SPSS version 26 (SPSS, Chicago, IL, USA).

Results

Clinical characteristics of patients with CZA-resistant KPC-producing CRKP

A total of 60 patients with CZA-resistant, KPC-producing CRKP were enrolled in the study. The clinical characteristics are shown in Table 1. Among these patients, 39 (65%) were male, and the median age was 73.5 years. Chronic kidney disease, stages 3–5, was the most common pre-existing condition (76.7%). Twelve cases (20%) were classified as colonization with CZA-resistant, KPC-producing CRKP (Fig. 1).

All patients acquired the infection nosocomially, with 16 patients (26.7%) contracting CZA-resistant, KPC-producing CRKP in the intensive care unit (ICU). A previous CRKP isolation was found in 47 patients (78.3%), and 42 patients (70%) had been exposed to CZA therapy within the previous 3 months. The median duration of CZA exposure was 13.5 days (IQR: 9.8–17.0). The median time interval from CZA exposure to the index culture was 27.5 days (IQR: 15.8–43.5).

Index cultures were most frequently collected from sputum ($n = 22$, 36.7%), followed by blood ($n = 19$, 31.7%). Twenty patients (33.3%) had polymicrobial isolations from the same culture site. The 14-day mortality was 20%, and the in-hospital mortality was 41.7%.

Comparison of clinical and microbiological characteristics in patients with and without KPC variant-producing strains

Table 1 compares the clinical and microbiological features between patients with KPC variant-producing strains and those with wild-type KPC strains. The time interval from admission to the index culture was significantly longer in the KPC variant group compared to the wild-type KPC group (56 days, IQR: 35.0-90.5 vs. 23 days, IQR: 13.0-82.5; $p = 0.027$). A higher proportion of patients in the KPC variant group had a previous CRKP isolation within 3 months compared to the wild-type KPC group ($n = 33$, 91.7% vs. $n = 14$, 58.3%; $p = 0.002$). The proportion of CZA exposure within 3 months before the index culture was also higher in the KPC variant group compared to the wild-type KPC group ($n = 33$, 91.7% vs. $n = 9$, 37.5%; $p < 0.001$). More KPC variant strains were isolated during CZA therapy compared to wild-type KPC strains ($n = 19$, 52.8% vs. $n = 2$, 8.3%; $p < 0.001$).

KPC variant-producing CRKP had a higher proportion of restored meropenem susceptibility (MIC ≤ 4 μg/mL) and high-level CZA resistance (CZA MIC ≥ 64 μg/mL) compared to wild-type KPC strains ($n = 26$, 72.2% vs. $n = 0$, 0%; $p < 0.001$; $n = 29$, 80.6% vs. $n = 1$, 4.2%; $p < 0.001$, respectively).

Genomic characteristics of CZA-resistant KPC-producing CRKP

A total of 60 CZA-resistant, KPC-producing strains were isolated from these 60 patients. Of these, 36 strains (60%) were KPC variant-producing, with 22 different KPC variant types identified. The most common variant was KPC-33 ($n = 12$), followed by KPC-35 ($n = 3$), KPC-44 ($n = 2$), and others, including KPC-14, KPC-144, KPC-170, KPC-31, KPC-41, KPC-46, KPC-53, KPC-71, KPC-78, KPC-90, and KPC-93, each represented by one isolate. The MLST

Table 1 Clinical and Microbiological characteristics of the patients with KPC variant and wild-type KPC-producing strains

	Overall population, *n* = 60	KPC variant, *n* = 36 (60%)	Wild-type KPC, *n* = 24 (40%)	*P* value
Male sex	39 (65)	24 (66.7)	15 (62.5)	0.740
Age	73.5 (60.0-85.5)	73.0 (59.3–83.3)	78.0 (63.5–90.0)	0.280
Comorbidities				
Solid malignancy	19 (31.7)	14 (38.9)	5 (20.8)	0.141
Hematologic malignancy	6 (10)	3 (8.3)	3 (12.5)	0.675
Metastatic malignancy	8 (13.3)	5 (13.9)	3 (12.5)	1
Cerebrovascular accident	12 (20)	8 (22.2)	4 (16.7)	0.746
Type 2 diabetes mellitus	20 (33.3)	13 (36.1)	7 (29.2)	0.576
Coronary artery disease	10 (16.7)	9 (25)	1 (4.2)	**0.040**
Chronic respiratory failure	36 (60)	22 (61.1)	14 (58.3)	0.830
Chronic obstructive lung disease	8 (13.3)	3 (8.3)	5 (20.8)	0.247
Chronic kidney disease, stage 3-5[a]	46 (76.7)	25 (69.4)	21 (87.5)	0.105
Immunocompromised status[b]	17 (28.3)	9 (25)	8 (33.3)	0.483
Recent surgery within 3 months	28 (46.7)	19 (52.8)	9 (37.5)	0.245
Before the index culture				
Previous CRKP isolation within 3 months	47 (78.3)	33 (91.7)	14 (58.3)	**0.002**
Previous exposure to CZA within 3 months	42 (70)	33 (91.7)	9 (37.5)	**< 0.001**
Previous duration of CZA therapy within 3 months	13.5 (9.8–17.0)	13.0 (9.5–17.0)	15.0 (10.0-16.5)	0.818
Time interval from last CZA exposure to the index culture	27.5 (15.8–43.5)	25.0 (14.5–41.0)	37.0 (19.0-49.5)	0.244
Time interval from admission to the index culture	49.5 (21.3–88.0)	56 (35.0-90.5)	23 (13.0-82.5)	**0.027**
Clinical samples origin				
Blood	19 (31.7)	7 (19.4)	12 (50)	**0.013**
Sputum	22 (36.7)	19 (52.8)	3 (12.5)	**0.002**
Urine	11 (18.3)	4 (11.1)	7 (29.2)	0.097
Abdominal fluid (ascites, bile)	8 (13.3)	6 (16.7)	2 (8.3)	0.457
The index culture				
ICU acquisition[c]	16 (26.7)	9 (25)	7 (29.2)	0.721
Infection	48 (80)	26 (72.2)	22 (91.7)	0.100
Septic shock[d] at the onset of infection	10 (16.7)	4 (11.1)	6 (25)	0.178
SOFA score	6.0 (4.0–10.0)	6.0 (4.0-9.8)	7.0 (4.0–10.0)	0.722
APACHE II score	24.0 (20.0-32.8)	23.0 (18.0-30.8)	27.0 (20.0–33.0)	0.267
Isolation during CZA use	21 (35)	19 (52.8)	2 (8.3)	**< 0.001**
Intubation when isolation	23 (38.3)	15 (41.7)	8 (33.3)	0.515
Renal replacement therapy when isolation	27 (45)	17 (47.2)	10 (41.7)	0.672
Polymicrobial isolations	20 (33.3)	12 (33.3)	8 (33.3)	1
Indwelling devices[e] during the index culture	34 (56.7)	22 (61.1)	12 (50)	0.395
Length of stay after isolation	26.5 (15-54.8)	27 (16-59.3)	19.5 (12-52.8)	0.308
Microbiological features				
Meropenem MIC ≤ 4 μg/mL	26 (43.3)	26 (72.2)	0 (0)	**< 0.001**
CZA MIC ≥ 64 μg/mL	30 (50)	29 (80.6)	1 (4.2)	**< 0.001**
Mortality				
7-day mortality	8 (13.3)	3 (8.3)	5 (20.8)	0.247
14-day mortality	12 (20)	4 (11.1)	8 (33.3)	**0.050**
In-hospital mortality	25 (41.7)	14 (38.9)	11 (45.8)	0.606

Data are presented as median (interquartile range) for continuous variables, and number (percent) for categorical variables. Statistically significant P values are highlighted in bold

Abbreviations: CZA, ceftazidime/avibactam; KPC, *Klebsiella pneumoniae* carbapenemase; CRKP, carbapenem-resistant *Klebsiella pneumoniae*; ICU, intensive care unit; SOFA score, Sequential Organ Failure Assessment score; APACHE II score, Acute Physiology and Chronic Health Evaluation II score; MIC, minimum inhibitory concentration

[a] Chronic kidney disease staging was based on estimated glomerular filtration rate

[b] Immunocompromised status was defined as neutropenia, acquired immunodeficiency syndrome, or receiving prednisolone over 20 mg/day for more than 7 days, immunosuppressants or cancer-related therapy for more than 4 weeks

[c] ICU-acquisition was defined as the index culture isolated after the patient admitted to ICU for more than 48 h

[d] Septic shock was defined as persistent hypotension requiring vasopressors to maintain a mean arterial pressure (MAP) ≥ 65 mmHg, with a serum lactate level > 2 mmol/L (18 mg/dL)

[e] Indwelling devices included central venous catheter, abdominal and pleural drain

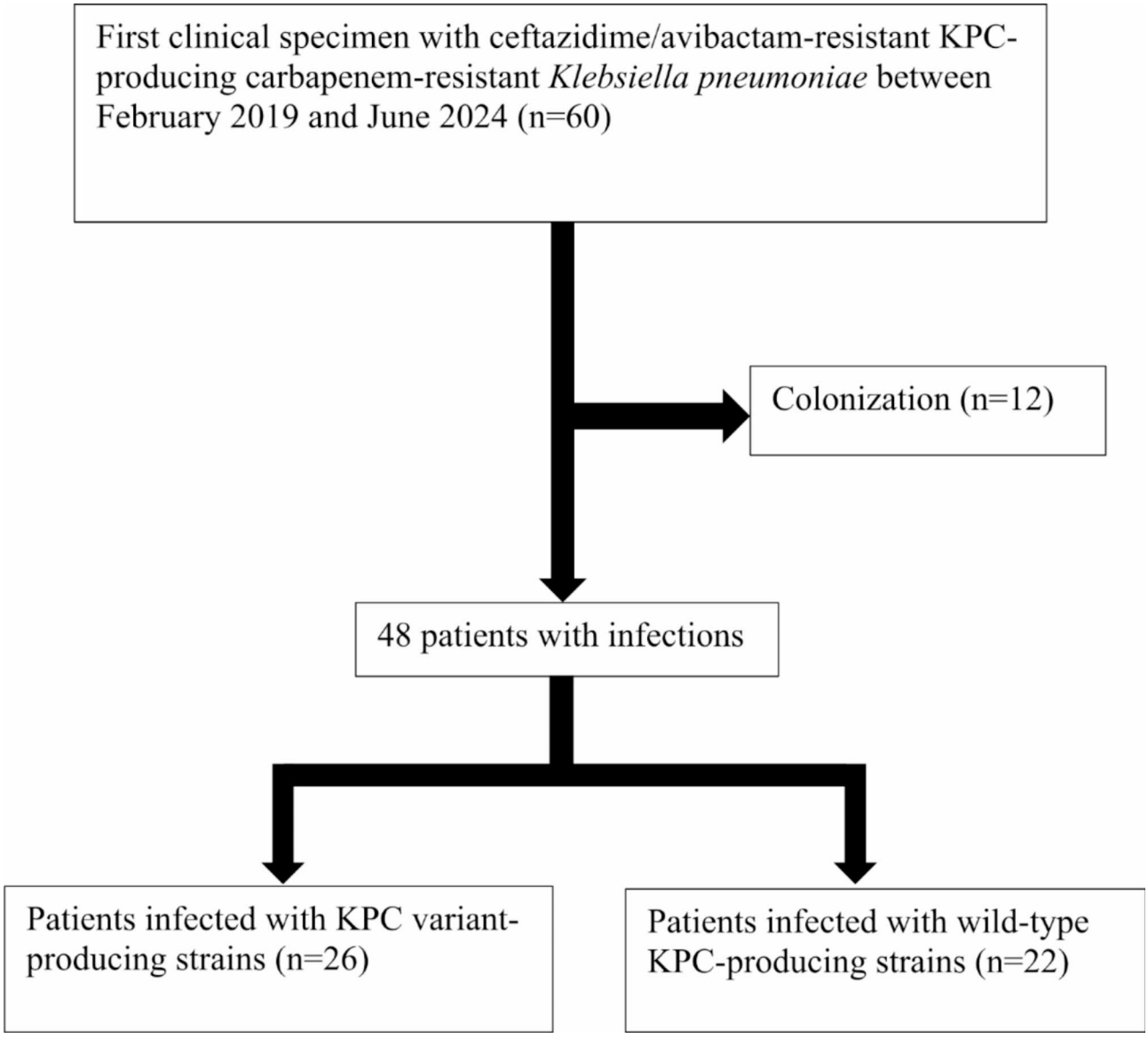

Fig. 1 Study population

showed all the KPC variant-producing strains belonged to ST11.

Eight novel KPC variants were identified, each displaying unique structural modifications. One variant involved a 2-amino acid (YG) deletion between positions 240 and 243 in KPC-2. Another variant featured the substitution D179Y in combination with T264S. There was a variant characterized by a 2-amino acid (EL) deletion between positions 165 and 168, coupled with an 8-amino acid (VYTRAPNK) duplication following Ambler amino acid position 269. Additionally, a variant exhibited the substitution D179Y combined with an 8-amino acid (NRAPNKDD) insertion between positions 271 and 272. Another variant had the substitution R164H in conjunction with a 2-amino acid (EL) deletion between positions 165 and 168. There was also a variant with a 7-amino acid (TSSPRAV) duplication after Ambler amino acid position 186. Another complex variant displayed the substitution D179Y combined with a 15-amino acid (YTRAPNKDD-KHSEAV) duplication after Ambler amino acid position 277. Finally, one variant was marked by a single amino acid (S) insertion between positions 182 and 183.

Most KPC variant isolates (26/36, 72.2%) exhibited decreased meropenem MICs (≤4 μg/mL). Other KPC variants had meropenem MICs ≥ 16 μg/mL, including strains producing KPC-33, KPC-44, KPC-144, KPC-31, KPC-41, KPC-90, and KPC-93. One novel KPC variant strain had a meropenem MIC of 8 μg/mL.

Table 2 Univariate and multivariate analyses of prognostic factors for 14-day mortality in the patients infected with CZA-resistant KPC-producing CRKP

	Univariate analysis		Multivariate analysis	
Prognostic factors	OR (95% CI)	P value	OR (95% CI)	P value
Septic shock at the onset of infection	6.60 (1.24–35.23)	**0.027**		
ICU-acquired infection	5.36 (1.14–25.26)	**0.034**		
SOFA score	1.23 (1.03–1.47)	**0.020**	1.31 (1.071–1.604)	**0.009**
APACHE II score	1.09 (0.99–1.20)	**0.069**		
KPC variants	0.30 (0.07–1.42)	0.129	0.14 (0.019–0.996)	**0.050**
Previous exposure to CZA within 3 months	0.21 (0.05-1.00)	**0.050**		

Abbreviations: CZA-resistant CRKP, ceftazidime/avibactam-resistant carbapenem-resistant *Klebsiella pneumoniae*; KPC, *Klebsiella pneumoniae* carbapenemase; ICU, intensive care unit; SOFA score, Sequential Organ Failure Assessment score; APACHE II score, Acute Physiology and Chronic Health Evaluation II score; OR, odds ratio; CI, confidence interval

Comparison of clinical characteristics in patients infected with and without KPC variant-producing strains

In total, 48 patients with infections were identified. Supplementary Table S1 compares the clinical characteristics between the KPC variant group and the wild-type KPC group. Disease severities were similar between the two groups. Hospital-acquired pneumonia was more common in the KPC variant group (69.2% vs. 22.7%; $p = 0.001$), while urinary tract infection was more common in the wild-type KPC group (11.5% vs. 40.9%; $p = 0.019$). Supplementary Table S2 presents the treatment and outcomes between the two groups. Overall, only 22 patients (47.8%) received appropriate therapy during the index infection, with tigecycline-based regimens being the most common appropriate definitive therapy in both groups. The proportion of appropriate therapy was similar between the two groups. The 14-day mortality was significantly higher in patients infected with wild-type KPC strains compared to those with KPC variants ($n = 8$, 36.4% vs. $n = 3$, 11.5%; $p = 0.041$), although in-hospital mortality was similar between the two groups. The Kaplan-Meier survival curve for 14-day mortality between wild-KPC group and KPC variants group is shown in Supplementary Figure S1.

Prognostic factors for 14-day mortality in patients with CZA-resistant KPC-producing CRKP infection

Two patients with early mortality within 48 h after the index culture were excluded. Among the 46 patients analyzed, 9 (19.6%) expired within 14 days after the onset of infection. Supplementary Table S3 compares the clinical and microbiological characteristics between 14-day survivors and non-survivors. KPC variants and predictors for 14-day mortality with a p-value < 0.1 in the univariate analysis were included in the multivariate analysis. Table 2 shows that the SOFA score at the onset of infection (Odds ratio, OR = 1.32; 95% confidence interval, CI = 1.071–1.604; $p = 0.009$) was a significant predictor of 14-day mortality, while the presence of KPC variants (OR = 0.14; 95% CI = 0.019–0.996; $p = 0.050$) was a protective factor.

Discussion

In our study, the 14-day and in-hospital mortality rates for patients with CZA-resistant, KPC-producing CRKP were 20% and 41.7%, respectively. We identified 22 different KPC variants from 36 KPC-producing strains, with bla_{KPC-33} being the most common genotype, followed by bla_{KPC-35}. Most KPC variant strains exhibited restored meropenem susceptibility (MIC ≤ 4 μg/mL) and high-level CZA resistance (CZA MIC ≥ 64 μg/mL). Patients with CZA-resistant, KPC variant-producing *K. pneumoniae* frequently had a history of CRKP isolation and CZA exposure within the preceding three months. Notably, these patients had a lower 14-day mortality rate compared to those infected with wild-type KPC-producing strains.

The literature on clinical and microbiological investigations of CZA-resistant, KPC-producing CRE is limited [2]. A recent review by Ding et al. identified only 18 cases of infections caused by KPC variant-producing Enterobacterales from the Pubmed database between 2017 and 2022, with *K. pneumoniae* being the most common pathogen ($n = 16$) and bla_{KPC-31} being the most frequent variant. All 18 patients had been treated with CZA before the isolation of KPC variant strains, with the time interval between CZA initiation and variant strain isolation ranging from 9 to 41 days [2]. However, this review did not address the characteristics of wild-type KPC isolates with CZA resistance. In our study, the median duration of prior CZA therapy was 13.5 days, and the median interval between the last CZA use and the index culture was 27.5 days. A significantly higher proportion of patients with KPC variant-producing CRKP had previous CZA exposure compared to the wild-type KPC group, supporting the hypothesis that CZA treatment is a risk factor for the emergence of KPC variant-producing strains. Notably, we found patients infected with KPC variants more frequently presented with pneumonia than those infected with wild-type KPC. Previous studies demonstrated the suboptimal ability of CZA to reach pharmacokinetics-pharmacodynamics target in lungs and might select the resistant strains more easily in the respiratory tract [8, 21]. The current findings corresponded to the previous

studies and suggested physicians should pay more attention to patients treated with CZA for pneumonia.

Our study revealed a high overall mortality rate among patients with CZA-resistant, KPC variant-producing *K. pneumoniae*. The in-hospital mortality rate of 45.8% observed in our study was higher than that from two retrospective studies in Italy. One single-center study between 2018 and 2022 by Boattini et al. showed in-hospital mortality of patients with CZA-resistant KPC-producing *K. pneumoniae* bacteremia was 22% [12]. The other 2-center study by Oliva et al. enrolled patients with CZA-resistant KPC-producing *K. pneumoniae* infections between 2019 and 2021, with in-hospital mortality 33.9% [13]. The high mortality from our data underscores the serious public health threat posed by CZA-resistant, KPC-producing *K. pneumoniae*. However, one Italian study [12] did not further determine KPC genotype of these *K. pneumoniae* strains and the other study [13] only presented KPC genotypes in 16 out of 59 strains. In our study, we determined the KPC genotype of all isolates, and incorporated the microbiological data for further investigation.

In the Italian study by Oliva et al., most survivors were treated with meropenem combination therapy, followed by meropenem-vaborbactam [13]. However, in Taiwan, where our study was conducted, novel antibiotics such as meropenem-vaborbactam, imipenem-relebactam, and cefiderocol, were not yet available during the study period. As a result, traditional drugs, including colistin, tigecycline, aminoglycosides, and meropenem combination therapy, were used for CZA-resistant, KPC-producing CRKP infections. Notably, our study found a low proportion of appropriate therapy, partly due to the unavailability of CZA MIC results at the initial antimicrobial susceptibility testing (AST) of the index culture in our laboratory. Clinicians often continued CZA treatment for CRKP infections until further MIC testing was requested in cases of suspected resistance. Our findings highlight the importance of repeating AST for newer β-lactams, as recommended by the Infectious Disease of Society America, to monitor the development of resistance and guide appropriate therapy [3]. The recent implementation of rapid antimicrobial susceptibility testing of CZA is also a promising tool in antimicrobial stewardship to help early appropriate therapies in target pathogen and subsequent acquired pathogen [22–25]. Currently, no consensus exists in international guidelines on the best treatment options for CZA-resistant, KPC-producing *K. pneumoniae* infections, underscoring the need for further research.

The prevalence of KPC variants vary with geographic location. One recent review reported that the USA and China ranked first and second with 63 and 59 types of KPC variants respectively until March 2023 [2]. As of June 2024, more than 200 KPC variants have been reported worldwide according to the National Center for Biotechnology Information Reference Sequences database [26]. Our study identified 22 types of KPC variants, including 8 novel ones, during the study period. Previous studies have shown that *K. pneumoniae* harboring KPC-2 with the D179Y mutation (known as KPC-33) tends to exhibit restored meropenem susceptibility [2, 10, 12, 13]. In our study, most KPC variant isolates had decreased meropenem MICs (≤ 4 μg/mL) and high-level resistance to CZA (MIC ≥ 64 μg/mL). In contrast, all wild-type KPC strains had meropenem MICs > 4 μg/mL, and most (23/24, 95.8%), including 17 KPC-2 strains and 6 KPC-3 strains, exhibited low-level resistance to CZA (MIC < 64 μg/mL). The possible mechanisms for CZA resistance in wild-type KPC strains may involve hyperexpression of the bla_{KPC} gene and loss of outer membrane porins, as suggested by the literature [9], although these mechanisms were not explored in our study. Interestingly, the 14-day mortality was significantly lower in patients infected with KPC variant-producing strains compared to those with wild-type KPC. We also identified KPC variants as independent factors associated with 14-day survivor. In a recent *in vitro* study, we observed that mutated KPC variants, such as KPC-33 and KPC-39, conferred high-level CZA resistance but were associated with significant fitness costs [27]. This fitness cost may partially explain the lower 14-day mortality observed in patients. However, further research is needed to confirm these preliminary findings.

The strength of our study lies in the comprehensive identification of KPC variant-producing strains and the clinical investigation of these cases. We identified up to 22 types of KPC variants, including 8 novel KPC variants, and found that most of these strains exhibited high-level CZA resistance and restored meropenem susceptibility. Nonetheless, our study has limitations. The retrospective design and single-center study limited the generalizability. We focused on the patients with nosocomial acquisition, which could not reflect all the patients at risk of CZA-resistant KPC-producing CRKP infections. Though we found a link between KPC variants and reduced 14-day mortality, the possibility of unmeasured confounders remained due to the retrospective nature and limited case numbers. Finally, we did not perform the whole genome sequencing among the KPC variant-producing strains, and the genetic context and clonal relationships were not clearly explored.

Conclusions

Our study found a high mortality rate in patients with CZA-resistant, KPC-producing CRKP. KPC variant-producing CRKP isolates tended to have restored meropenem susceptibility (≤ 4 μg/mL) and high-level CZA

resistance (≥64 μg/mL). KPC variants were associated with lower 14-day mortality. Further research is necessary to better understand this emerging public health threat, especially given the increasing identification of CZA-resistant, KPC-producing CRKP worldwide and the associated high mortality.

Abbreviations

CZA	Ceftazidime/avibactam
KPC	Klebsiella pneumoniae carbapenemase
MIC	Minimal inhibitory concentrations
CRE	Carbapenem-resistant Enterobacterales
CRKP	Carbapenem-resistant Klebsiella pneumoniae
OXA-48	Oxacillinase-48
MBL	Metallo-β-lactamases
SOFA score	Sequential Organ Failure Assessment score
APACHE II score	Acute Physiology and Chronic Health Evaluation II score
MAP	Mean arterial pressure
AIDS	Acquired immunodeficiency syndrome
CLSI	Clinical and Laboratory Standards Institute
PCR	Polymerase chain reaction
IQR	Interquartile ranges
ICU	Intensive care unit
OR	Odds ratio
CI	Confidence interval

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-025-00797-5.

Supplementary Material 1

Acknowledgements
The preliminary data of this study were submitted to IDweek 2024 as a paper poster. We sincerely thank Medical Science and Technology Building of the Taipei Veterans General Hospital for providing experimental space and facilities.

Author contributions
S. Y. L. performed the data analysis and wrote the original draft of the manuscript. S. H. C., C. C., C. H. J., and H.L.H performed data collection. Y. T. L. contributed to study design, data collection, funding acquisition, revising the manuscript. L.C., Y. C. H. and S. Y. L. directly accessed and verified the underlying data. All authors reviewed and approved the final manuscript.

Funding
This work was supported by grants from the Ministry of Science and Technology (MOST 111-2314-B-A49-044), National Science and Technology Council (NSTC 112-2314-B-A49-029) in Taiwan, the Taipei Veterans General Hospital (V112C-102, V113C-073 and V114C-047). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Ethical approval and consent to participate
This study was approved by the Institutional Review Board of Taipei Veterans General Hospital (2024-01-004BC) and informed consent was waived.

Conflict of interest
The authors declare no competing interests.

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Received: 11 February 2025 / Accepted: 8 April 2025
Published online: 18 April 2025

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