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Different Effects of Antibiotics on *Klebsiella pneumoniae* and *Escherichia coli* Resistance Induced by Antibiotics: A Retrospective Study from China

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Objective: The main objective was to assess the correlation between antibiotic use and carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and carbapenem-resistant *Escherichia coli* (CREC) induction by antibiotics.
Materials and Methods: A retrospective cohort study was conducted from January 2017 to December 2020. This study included patients with *K. pneumoniae* and *E. coli.* Kaplan–Meier analysis and Cox proportional hazard model were used to estimate the hazard of carbapenem-resistant Enterobacterales (CRE), whereas restricted cubic spline regression was used to visualize the hazard of CRE by antibiotics at different doses.
Results: Two thousand fifty-six *K. pneumoniae* patients and 3,243 *E. coli* patients were included. After Cox proportional hazard model analysis, carbapenems or 1st-cephalospoins or penicillin monotherapy, male and ICU admission were associated with CRKP. CREC was associated with quinolone monotherapy. Time-to-event analysis indicated that carbapenem, β-lactamase inhibitor mixtures, and quinolones were associated with higher 30-day CRKP hazards than other antibiotics ($\chi^2 = 33.670$, $p < 0.001$). Further restricted cubic spline regression analysis found that the hazard of CRKP induction decreased with the increased dose of β-lactamase inhibitor mixtures, but there was no significant change in the hazard ratio of CRKP induction with the increased dose of quinolones. Moreover, there was an obvious characteristic of ''parabolic curve'' for the hazard of CREC induction due to β-lactamase inhibitor mixtures, and the hazard value gradually increased with the dose, reached the maximum at 24 g, and finally gradually decreased from 26 g.
Conclusions: Rational use of antibiotics should be implemented and antimicrobial stewardship policies should be adjusted according to the characteristics of each hospital.

Keywords: *Klebsiella pneumoniae*, *Escherichia coli*, induction effects, antibiotic

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Background

Over recent years, antimicrobial resistance (AMR) among Enterobacterales has become a serious public health problem in the world. The AMR greatly limits therapeutic options, resulting in higher morbidity, mortality and huge economic burden.[1]

Carbapenem is a β-lactam antibiotic with a wide antibacterial spectrum and strong antibacterial activity, and it is often used as a last resort in the treatment of multidrug-resistant Gram-negative Enterobacterales infection.[2,3] However, the incidence of carbapenem-resistant Enterobacterales (CRE) has constantly increased over past years, posing a challenge for clinical antimicrobial chemotherapy and hospital infection control.[4–6] In China, *Klebsiella pneumoniae* and *Escherichia coli* were the most common Enterobacterales according to CHINET surveillance in 2018, which could cause nosocomial infections, such as pneumonia, urinary tract infection, and catheter-related bloodstream infection.[7] Previous studies had reported that CRE was associated with high mortality.[8–10]

Rational use of antibiotics can effectively treat bacterial infections and reduce the burden to patients. However, unreasonable use of antibiotics will increase the selective pressure of antimicrobials commonly used, which is one of the important factors leading to AMR, and some evidence showed that increased use of antibiotics could lead to the emergence of AMR.[11,12] Numerous studies reported the significant relationship between antimicrobial consumption and CRE prevalence, but whether antibiotics at different doses contribute to CRE was not yet certain.

It was necessary to understand the risk factors of CRE and evaluate the correlation between antibiotic dose and CRE, to provide evidence for developing a plan to reduce selective pressure imposed by antimicrobial agents in the future. Accordingly, we conducted this study to assess the correlation between antibiotic use and isolation of carbapenem-resistant *E. coli* (CREC) and carbapenem-resistant *K. pneumoniae* (CRKP) in a large cohort.

Materials and Methods

Design and subjects

A retrospective analysis was performed in Sichuan Academy of Medical Sciences and Sichuan People's Hospital, a 3,270-bed teaching hospital in Western China for the period January 2017 to December 2020. This study population comprised all patients with *K. pneumoniae* or *E. coli* cultured from any of the clinical specimens. This study was approved by the Ethics Committee of Sichuan Academy of Medical Sciences and Sichuan People's Hospital. The Review Board exempted the requirement for informed consent because of the retrospective study and the absence of a negative impact on the patients.

Microbiology

Drug sensitivity test and result interpretation referred to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) 2017.[13] Agar dilution method using Mueller Hinton agar was used for the general bacterial susceptibility test. Disk-diffusion synergy test recommended by CLSI was used to screen and detect *E. coli* and *K. pneumoniae*. The automated instrument used VITEK 2 (BioMerieux, France) compact automatic microbial identification and drug sensitivity analysis system. The disk-diffusion synergy test was the Kirby-Bauer method, and the source of the drug sensitive paper was purchased from BBL and Oxoid.

Quality control was performed according to CLSI 2017 requirements during each test and every day.[13] The quality control strains (purchased from the Clinical Laboratory Center of the Ministry of Health, PRC) included *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 35218.

Outcomes and definition

CRKP isolation and CREC isolation were the primary outcome of this study. Carbapenem resistance is defined as an minimum inhibitory concentration (MIC) of ≥2 μg/mL for ertapenem or an MIC of ≥4 μg/mL for meropenem or imipenem or dolipenem, according to the guidelines of CLSI 2017.[13]

Confounding factors

Potential confounders were selected based on previous literature,[14–16] and they included age, gender, tumor, liver failure, kidney failure, heart failure, respiratory failure, diabetes, hypertension, chronic obstructive pulmonary disease, hemodialysis, venous catheterization, mechanical ventilation, urinary catheterization, tracheotomy, surgery, ICU admission now or in the past, and community infections.

Data collection

All the data were collected from the Data Management Platform (DMP) of the hospital, which is composed of Hospital Information System (HIS), Laboratory Information System (LIS), Nursing Information System (NIS), and Nosocomial Infection Surveillance System (NISS). Each system transmitted data in real time on the platform (Supplementary Fig. S1). To accomplish the study objectives, the data management was carried out by a full-time health statistics professional of HAI surveillance in the drug-resistant bacteria management module of NISS.

Statistical analysis

Statistical analysis of the data was performed by using STATA version 20.0 (StataCorp., College Station, TX, USA) and SPSS version 23.0 (IBM Corporation, Armonk, NY, USA). Data were summarized by using the mean and standard deviation for normally distributed variables. Categorical variables were expressed in absolute numbers and percentages. We used both univariate and multivariate methods to analyze the data. Binary outcomes were tested by using the χ^2 test, and continuous data were compared by using the *T*-test.

Kaplan–Meier analysis and Cox proportional hazard model were used to estimate the hazard of CRE in the time-to-event analysis. For the time-to-event analysis, the endpoints in this study were 30 days from antimicrobial therapy until CRE detected from any isolate. Restricted cubic spline regression was used to visualize the hazard of CRE by antibiotics at different doses.

To assess the hazard of CRE induction by antibiotics, the data were gradually analyzed in two parts. First, in the COX

proportional hazard model, we evaluated the CRE induction effect of various commonly used antibiotics on the basis of excluding other confounding factors. Second, to analyze the dose–response relationship between the antibiotics and the CRE induction effect, we fit the curve of the specific antibiotics with the strongest CRE induction effect on the basis of excluding other confounding factors. p Values below 0.05 were considered significant.

Data availability statement

All data generated and analyzed during this study are included in this article.

Results

Patient inclusion

From January 1, 2017 to December 31, 2020, a toal of 6,339 inpatients with Enterobacterales cultured from clinical specimens, including 2,056 patients with *K. pneumoniae* cultured and 3,243 patients with *E. coli* cultured, as Figure 1 shows.

Clinical characteristics

Among 2,056 patients with *K. pneumoniae* and 3,243 patients with *E. coli*, there were 308 (14.98%) CRKP and 183 (5.64%) CREC, respectively, whereas the remaining were cases with carbapenem-sensitive Enterobacterale.

Univariate analysis (Table 1) shows the following factors to be associated with a higher risk of CRKP occurrence: male, urinary cannula, vascular cannula, mechanical ventilation, tracheotomy, blood transfusion or use of blood products, respiratory failure, ICU admission now or in the past, and community infections. Moreover, the following factors are shown to be associated with a higher risk of CREC occurrence: male, urinary cannula, vascular cannula, mechanical ventilation, blood transfusion or use of blood products, respiratory failure, heart failure, ICU admission now or in the past, and community infections.

Antimicrobial therapy before CRE were detected

The antimicrobial therapy before the detection of bacteria in the patient's clinical specimens is shown in Table 2. There is a significant difference between the occurrence of CSPK and CRKP in antimicrobial therapy among monotherapy, combination therapy, and unused ($\chi^2 = 90.667$, $p < 0.001$), and the proportion of combination antibacterial therapy in patients with CRKP is relatively high (181/308, 58.77%).

Moreover, there is a significant difference between these two groups in monotherapy among antibiotics used ($\chi^2 = 38.851$, $p < 0.001$). Among CSKP and CRKP patients, the proportion of patients using β-lactamase inhibitor mixtures is relatively high: (252/575, 43.83%) and (50/74, 67.57%), respectively.

There is a significant difference in antibacterial therapy between the CSEC group and the CREC group ($\chi^2 = 33.021$, $p < 0.001$), and the proportion of combination antibacterial therapy in patients with CREC is relatively high (75/183, 40.98%). Moreover, there is also a significant difference in monotherapy between these two groups ($\chi^2 = 26.824$, $p < 0.001$), Among CSEC and CREC patients, the proportion of patients using β-lactamase inhibitor mixtures is relatively high: (190/718,26.46%) and (21/38, 55.26%), respectively.

Thirty-day hazard of CRE induction by antibiotics

The results of the predicted time-to-event probability diagram indicate that the Cox proportional hazard hypothesis is valid (Supplementary Fig. S2). Omnibus Tests of model

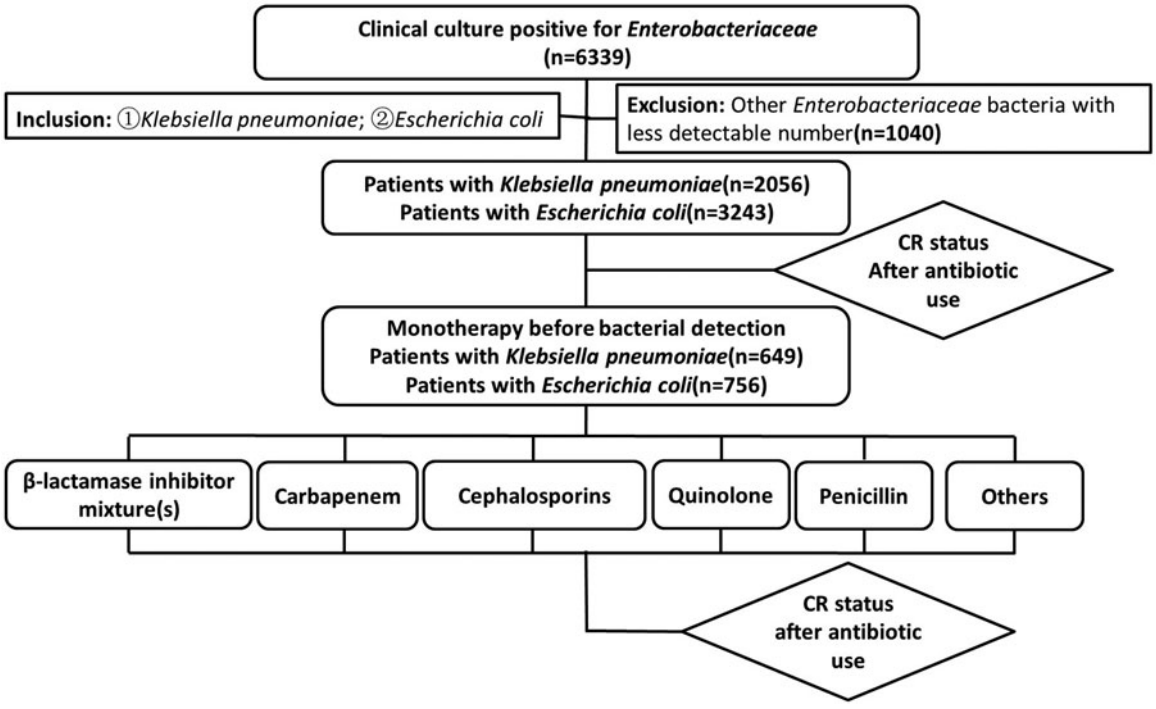

FIG. 1. Flow chart of patients' inclusion.

Table 1. Baseline Characteristics

Variables	*CSKP* (n = *1,748*), n *(%)*	*CRKP* (n = *308*), n *(%)*	χ^2	p	*CS-E. coli* (n = *3,060*), n *(%)*	*CR-E. coli* (n = *183*), n *(%)*	χ^2	p
Male	1,142 (65.33)	232 (75.32)	11.796	0.001	1,248 (40.78)	112 (61.20)	29.563	0.000
Urinary cannula	759 (43.42)	173 (56.17)	17.171	0.000	1,084 (35.42)	92 (50.27)	16.471	0.000
Vascular cannula	550 (31.46)	122 (39.61)	7.897	0.005	717 (23.43)	78 (42.62)	34.369	0.000
Mechanical ventilation	479 (27.40)	122 (39.61)	18.864	0.000	360 (11.76)	44 (24.04)	23.873	0.000
Tracheotomy	157 (8.98)	44 (14.3)	8.352	0.004	79 (2.58)	8 (4.37)	2.119*	0.145
ICU admission now or in the past	784 (44.85)	193 (62.66)	33.310	0.000	797 (26.05)	68 (37.16)	10.903	0.001
Hemodialysis*	15 (0.86)	2 (0.65)	0.001	0.975	10 (0.33)	1 (0.55)	0.000	1.000
Blood transfusion or use of blood products	651 (37.24)	168 (54.54)	32.711	0.000	835 (27.29)	97 (53.01)	55.767	0.000
Hypertension	707 (40.45)	140 (45.45)	2.711	0.100	1,101 (35.98)	75 (40.98)	1.870	0.171
Diabetes	484 (27.69)	86 (27.92)	0.007	0.933	806 (26.34)	48 (26.23)	0.001	0.974
Chronic obstructive pneumonia	192 (10.98)	36 (11.69)	0.132	0.717	173 (5.65)	14 (7.65)	1.267	0.260
Tumor	270 (15.50)	48 (15.58)	0.001	0.971	563 (18.40)	31 (16.94)	0.246	0.620
Liver failure	27 (1.54)	9 (2.92)	2.888	0.089	36 (1.18)	3 (1.64)	0.044*	0.835
Kidney failure	178 (10.18)	37 (12.01)	0.936	0.333	217 (7.09)	19 (10.38)	2.772	0.096
Heart failure	137 (7.84)	27 (8.77)	0.308	0.579	181 (5.91)	19 (10.38)	5.955	0.015
Respiratory failure	282 (16.13)	92 (29.87)	33.207	0.000	202 (6.60)	28 (15.30)	19.831	0.000
Surgery	1,340 (76.66)	241 (78.25)	0.372	0.542	2,195 (71.73)	137 (74.86)	0.838	0.360
Infection status before CRE detection	451 (25.80)	104 (33.77)	8.430	0.004	679 (22.19)	52 (28.42)	3.833	0.050
Principal diagnosis (ICU-10 code)								
Certain infectious diseases and parasites (A00–B99)	142 (8.12)	26 (8.44)	68.783	0.000	215 (7.03)	12 (6.56)	38.511	0.005
Tumor (C00–D48)	206 (10.99)	30 (9.74)			442 (14.44)	38 (20.77)		
Blood and hematopoietic diseases and certain diseases involving immune mechanisms (D50–D89)	7 (0.22)	2 (0.65)			17 (0.56)	1 (0.55)		
Endocrine, nutritional, and metabolic diseases (E00–E90)	45 (2.57)	1 (0.32)			122 (3.99)	6 (3.28)		
Mental and behavioral disorders (F00–F99)	31 (1.77)	7 (2.77)			84 (2.75)	4 (2.19)		
Nervous system diseases (G00–G99)	69 (3.95)	17 (5.52)			122 (3.99)	6 (3.28)		
Eye and appendage diseases (H00–H59)	0 (0.00)	0 (0.00)			3 (0.10)	0 (0.00)		
Ear and mastoid diseases (H60–H95)	0 (0.00)	0 (0.00)			2 (0.07)	0 (0.00)		
Circulatory diseases (I00–I99)	299 (17.11)	42 (13.64)			276 (9.02)	16 (8.74)		
Respiratory diseases (J00–J99)	367 (21.00)	94 (30.52)			246 (8.04)	26 (14.21)		
Digestive diseases (K00–K93)	199 (11.38)	23 (7.47)			316 (10.33)	20 (10.93)		
Skin and subcutaneous tissue diseases (L00–L99)	22 (1.26)	3 (0.97)			51 (1.67)	2 (1.09)		
Musculoskeletal system and connective tissue diseases(M00–M99)	28 (1.60)	1 (0.32)			103 (3.37)	7 (3.83)		
Genitourinary diseases (N00–N99)	128 (7.32)	19 (6.17)			661 (21.60)	26 (14.21)		
Pregnancy, childbirth, and puerperium (O00–O99)	3 (0.17)	0 (0.00)			74 (2.42)	0 (0.00)		
Diseases that originated in the perinatal period (P00–P96)	16 (0.92)	17 (5.52)			56 (1.83)	0 (0.00)		
Congenital malformations, deformation, and chromosomal abnormalities (Q00–Q99)	5 (0.29)	1 (0.32)			15 (0.49)	0 (0.00)		
Abnormal symptoms, signs, clinical and laboratory results, and cannot be classified in other categories (R00–R99)	15 (0.86)	0 (0.00)			21 (0.69)	0 (0.00)		
Injury, poisoning, and other external pathogenic factors (S00–T98)	130 (7.44)	24 (7.79)			139 (4.54)	12 (6.56)		
External causes of illness and death (V01–V98)	36 (2.06)	1 (0.32)			95 (3.10)	7 (3.83)		
Age (M ± SD)[a]	66.51 ± 24.67	64.67 ± 26.26	1.194	0.233	62.08 ± 22.75	63.62 ± 23.05	−0.887	0.375

Data are no. (%) of objects. [a]Student *T*-test.

*Chi-square test with continuous correction.

CSKP, carbapenem-sensitive *Klebsiella pneumoniae*; CRKP, carbapenem-resistant *K. pneumoniae*; CS-*E. coli*, carbapenem-sensitive *Escherichia coli*; CS-*E. coli*, carbapenem-resistant *E. coli*; SD, standard deviation.

Table 2. Basic Situation of Antimicrobial Therapy

Variables	*CSKP,* n *(%)*	*CRKP,* n *(%)*	χ^2	p	*CS-E. coli,* n *(%)*	*CR-E. coli,* n *(%)*	χ^2	p
Antibacterial therapy								
Monotherapy	575 (32.89)	74 (24.03)	90.667	0.000	718 (23.46)	38 (20.77)	33.021	0.000
Combination therapy	545 (31.18)	181 (58.77)			693 (22.65)	75 (40.98)		
Unused	628 (35.93)	53 (17.21)			1,649 (53.88)	70 (38.25)		
Monotherapy								
β-lactamase inhibitor mixtures	252 (43.83)	50 (67.57)	38.851	0.000	190 (26.46)	21 (55.26)	26.824	0.000
Carbapenem	8 (1.39)	4 (5.41)			5 (0.70)	0 (0.00)		
1st-Cephalosporins	125 (21.74)	5 (6.76)			119 (16.57)	0 (0.00)		
2nd-Cephalosporins	40 (6.96)	2 (2.70)			93 (12.95)	4 (10.53)		
3rd-Cephalosporins	31 (5.39)	1 (1.35)			81 (11.28)	7 (18.42)		
Quinolone	40 (6.96)	10 (13.51)			176 (24.51)	5 (13.16)		
Penicillin	65 (11.30)	2 (2.70)			49 (6.82)	1 (2.63)		
Others	14 (2.43)	0 (0.00)			5 (0.70)	0 (0.00)		

Data are no. (%) of objects.

coefficients show that the Cox proportional hazards model for CRKP patients has obtained a statistically significant result ($\chi^2 = 40.003$, $p < 0.001$), but the model for CREC patients has not ($\chi^2 = 15.555$, $p = 0.158$).

In the COX proportional hazard model (Table 3), CRKP patients show association with carbapenem monotherapy (hazard ratio [HR], 3.252; 95% confidence interval [CI], 1.159–9.124), 1st-cephalospoins monotherapy (HR, 0.219; 95% CI, 0.085–0.564), penicillin monotherapy (HR, 0.191; 95% CI, 0.046–0.787), male (HR, 1.886; 95% CI, 1.059–3.360), and ICU admission now or in the past (HR, 1.803; 95% CI, 1.117–2.910). The CREC patients show only an association with quinolone monotherapy.

Time-to-event analysis (Fig. 2) indicates that the administration of carbapenem, β-lactamase inhibitor mixtures, and quinolones are associated with higher 30-day CRKP isolation hazards than other antibiotics. Log-rank test results show that the differences are statistically significant ($\chi^2 = 33.670$, $p < 0.001$) (Fig. 2A). Moreover, the 30-day CREC isolation hazards are significantly higher in patients with administration of β-lactamase inhibitor mixtures and 3rd-cephalosporins than other antibiotics ($\chi^2 = 17.634$, $p = 0.003$) (Fig. 2B).

Dose–response relationship between the hazard of CRE induction and antibiotics

In restricted cubic spline regression analysis (Figs. 3 and 4), the fitted curve shows that the hazard of CRKP induction decreases with the increased dose of β-lactamase inhibitor mixtures, but there is no significant change in the HR of CRKP induction with the increased dose of quinolones (Fig. 3).

Moreover, there is an obvious characteristic of ''parabolic curve'' for the hazard of CREC induction due to β-lactamase inhibitor mixtures, and the hazard value gradually increases with the dose, reaches the maximum at 24 g, and finally gradually decreases from 26 g. However, there is no significant change in the HR of CREC induction with the increased dose of 3rd-cephalosporins (Fig. 4).

Discussion

Carbapenems are the most effective drugs for the treatment of severe infections with gram-negative bacteria due to their broad antimicrobial spectrum and high stability for hydrolysis by most β-lactamases, including extended-

Table 3. Equation Parameters of Cox Proportional Hazard Model

	Klebsiella pneumoniae		Escherichia coli	
Covariate	*HR*	*95% CI*	*HR*	*95% CI*
Antibacterial monotherapy (control = β-lactamase inhibitor mixtures)				
Carbapenem	3.252	1.159–9.124	0.995	/
1st-Cephalosporins	0.219	0.085–0.564	0.964	/
2nd-Cephalosporins	0.383	0.092–1.594	0.099	0.138–1.187
3rd-Cephalosporins	0.247	0.034–1.797	0.571	0.331–1.838
Quinolone	1.196	0.601–2.382	0.030	0.128–0.903
Penicillin	0.191	0.046–0.787	0.195	0.035–1.978
Others	0.000	/	0.991	/
Gender (control = female)	1.886	1.059–3.360	/	/
Blood transfusion or use of blood products	1.535	0.938–2.513	/	/
ICU admission now or in the past	1.803	1.117–2.910	/	/

/There are no data in the model fitting results or the variable is not included in the model.
CI, confidence interval; HR, hazard ratio.

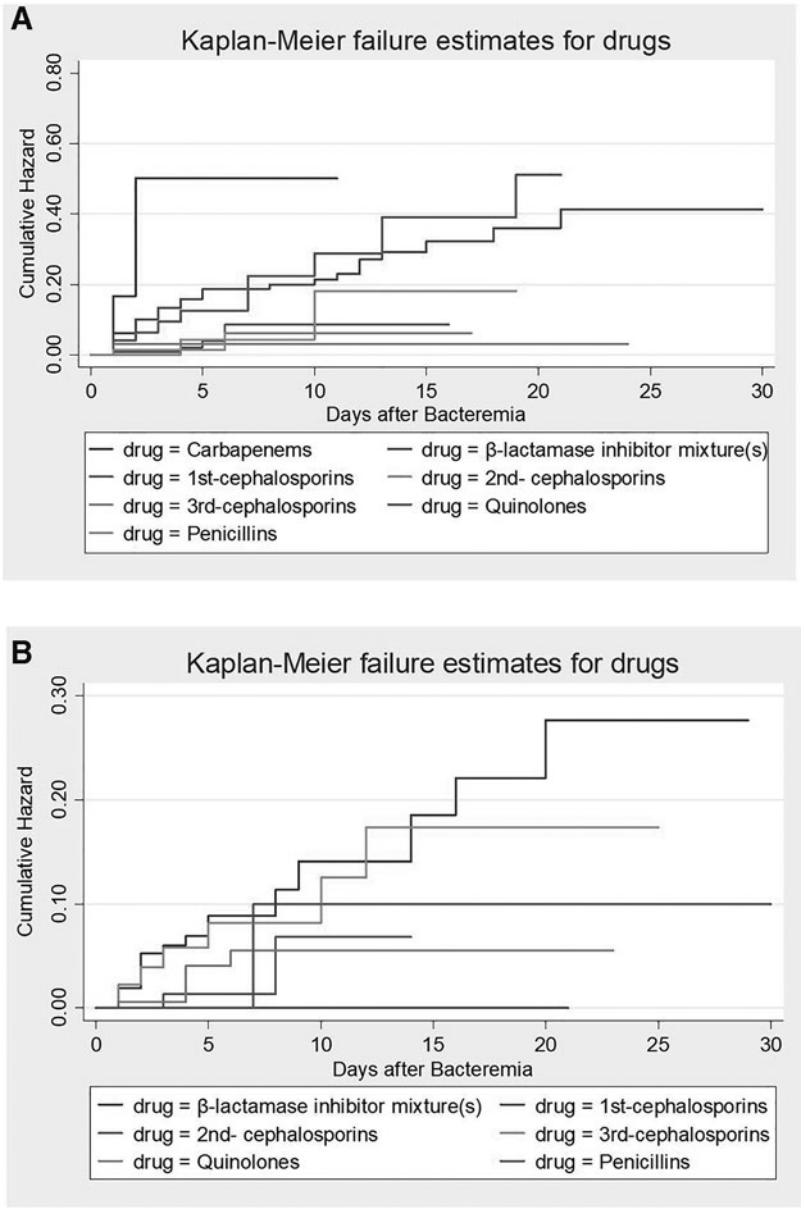

FIG. 2. Results of Kaplan–Meier analysis: **(A)** comparison of CRKP induction between seven antibiotics; **(B)** comparison of CREC induction between seven antibiotics. CREC, carbapenem-resistant *Escherichia coli*; CRKP, carbapenem-resistant *Klebsiella pneumoniae*.

spectrum β-lactamases (ESBLs) and AmpC cephalosporinases.[17] However, unreasonable use of antibiotics leads to the emergence of CRE, which is becoming more and more serious, and the treatment option is limited.[7,18,19] Therefore, understanding the hazard factors for antibiotic-induced CRE is very important in the early selection of the empirical antibiotic program.

In this study, we explored the relationship between the use of antibiotics and the occurrence of CRKP and CREC, respectively. Compared with CSE, the risk factors of CRE in clinical samples were male, urinary cannula, vascular cannula, mechanical ventilation, blood transfusion or use of blood products, respiratory failure, and ICU admission.

Previous studies confirmed that antibiotic exposure was a risk factor for CRE infection.[20,21] In our study, the first significant finding was that the difference in antibacterial therapy among monotherapy, combination therapy, and unused was significant between the CSKP group and the CRKP group, and the proportion of combined antibacterial therapy in the CRKP group was higher. Increased exposure to one antibiotic increased the effect of exposure to other antibiotics, at the risk of CRKP infection.[22] Therefore, combined use of antibiotics would increase the selection pressure of antibiotics, allowing carbapenem-resistant bacteria to produce various carbapenem-resistant mechanisms.[23] This result was consistent with that in the CSEC group and the CREC group. During the study, β-lactamase inhibitor mixtures were the most commonly used antibiotics.

The second significant finding was that carbapenem, β-lactamase inhibitor mixtures, and quinolones were associated with higher 30-day CRKP isolation hazards than other antibiotics in the time-to-event analysis. Among the antibiotics, carbapenems comprised the most principal hazard factor, which was consistent with previous studies.[2,24–30] The use of carbapenem may promote the production of carbapenemase, such as *K. pneumoniae* carbapenemase and metallo-β-lactamases, which could increase the production of CRE.[31]

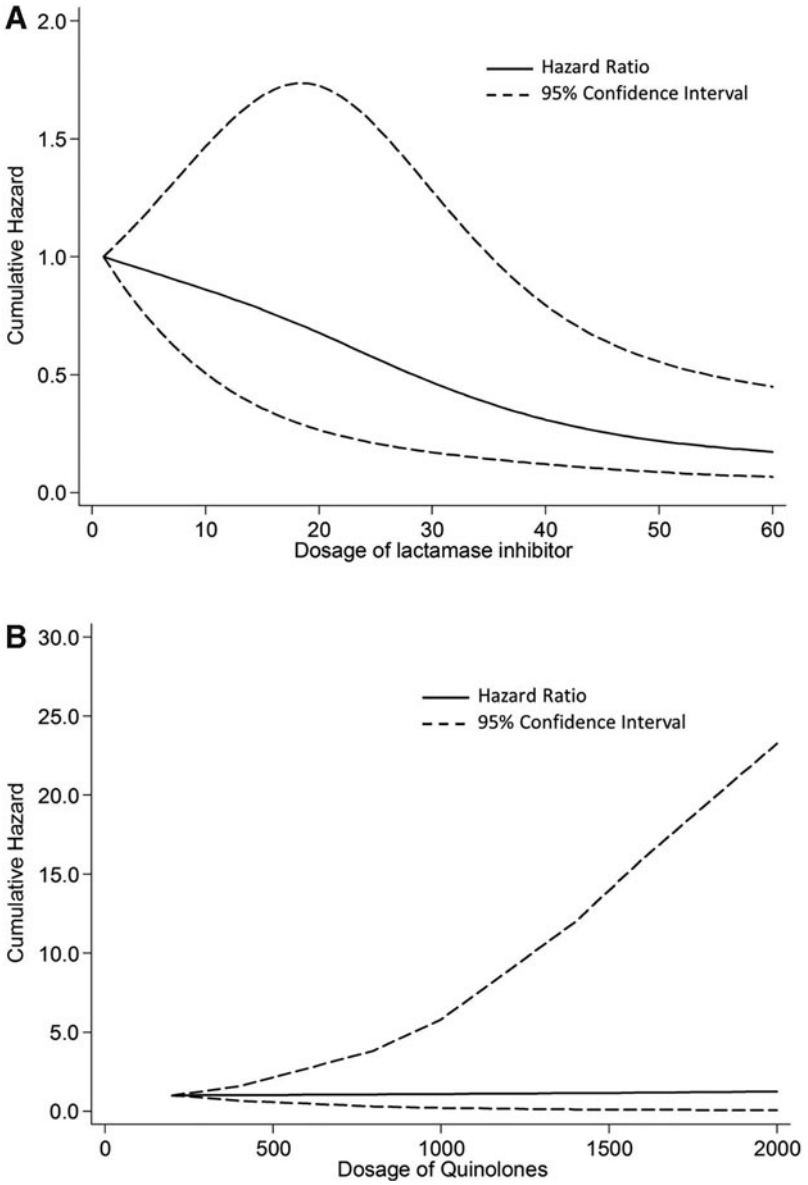

FIG. 3. Results of restricted cubic spline regression on *Klebsiella pneumoniae*: **(A)** The hazard of CRKP induction decreased with the increased dose of β-lactamase inhibitor mixtures; **(B)** There was no significant change in the HR of CRKP induction with the increased dose of quinolones. HR, hazard ratio.

Other mechanisms of carbapenem resistance include outer membrane porin expression loss combined with ESBLs and AmpC enzyme, change of antimicrobial target, and high expression of efflux pump.[32–34] The result suggested that the restriction of carbapenems was associated with a significant reduction in the incidence of CRKP. However, for CREC, the 30-day hazards were significantly higher in patients with β-lactamase inhibitor mixtures and 3rd-cephalosporins than other antibiotics.

Last but not least, we found that the hazard of CRKP induction decreased with the increased dose of β-lactamase inhibitor mixtures, but there was no significant change in the HR of CRKP induction with the increased dose of quinolones or carbapenems. The result indicated that the risk of CRKP induction was higher in low-dose β-lactamase inhibitor mixtures exposure. Therefore, we suggested that adequate dose of β-lactamase inhibitor mixtures should be used in the treatment of these patients. However, there was no relevant research, and further studies were necessary to explore the mechanism.

We also found that increasing the use of carbapenem or quinolones did not lead to a significant increase in the resistance of carbapenem among Enterobacterales, which was similar to a few previous studies.[35–37] However, some studies showed that carbapenem or fluoroquinolone use had a positive relationship with the incidence of CRE.[36,38] The result was still controversial, which might be explained by the influence of multiple interactive factors that had an impact on the induction of CRE. As a preceding result, the hazard of CRKP induction decrease might be responsible for the increasing dose of β-lactamase inhibitor mixtures.

Moreover, there was an obvious characteristic of a ''parabolic curve'' for the hazard of CREC induction due to β-lactamase inhibitor mixtures, and the hazard value gradually increased with the dose, reached the maximum at 24 g, and finally gradually decreased from 26 g. In other words, with the increasing use of β-lactamase inhibitor mixtures, the risk of CREC induction increased and reached the maximum when the dose of β-lactamase inhibitor mixtures reached 24 g, but it gradually decreased when the dose exceeded 26 g.

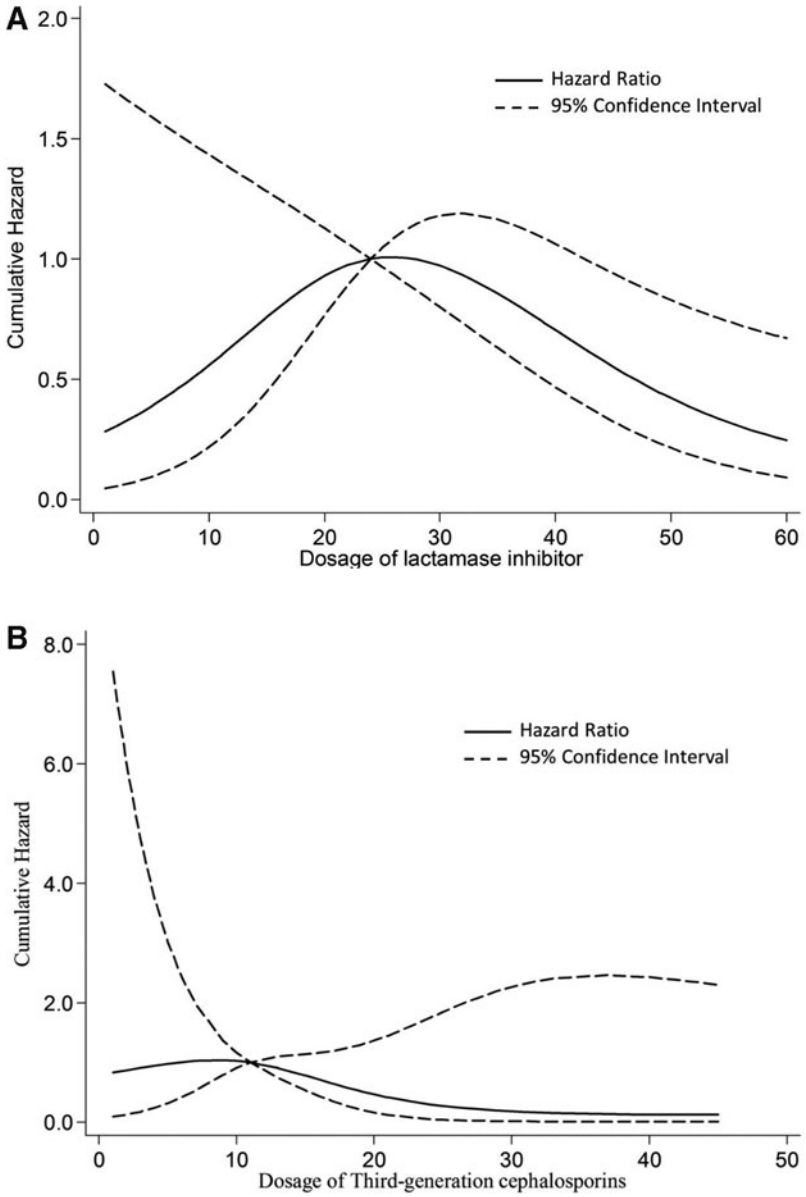

FIG. 4. Results of restricted cubic spline regression on *Escherichia coli*: **(A)** There was a clear threshold (24 g) for the hazard of CREC induction due to β-lactamase inhibitor mixtures; **(B)** There was no significant change in the HR of CREC induction with the increased dose of 3rd-cephalosporins.

However, no existing study reported this correlation. This result may explain why the incidence of CREC was low in CRE. Although antibiotic exposure may lead to clinically significant antibiotic resistance, the effects are not consistent and vary with the organism and antibiotic resistance mechanism. Large sample studies were needed to further clarify the mechanism of the effect of β-lactamase inhibitor mixture doses on CRE.

In this study, β-lactamase inhibitor mixtures had different drug resistance, inducing effects on CRKP and CREC, which suggested that the maximum dose should not be blindly pursued in clinical medication. We should choose the appropriate treatment plan according to the specific characteristics of microbial distribution, or adjust the antimicrobial treatment strategy in real time according to the characteristics of microbial distribution, which raised higher requirements for the rational use of antibiotics.

There were several potential limitations in our study. First, this study was a retrospective design conducted in a large tertiary A-level hospital, not multicenter research. Second, due to the low prevalence of CRE and the two types of CRE that were analyzed according to the use of antibiotics, the sample size was not large enough. Third, in our study, patients with CRKP were less likely to use carbapenems before detection of CRKP.

So, we found that there was no significant change in the HR of CRKP induction with the increased dose of carbapenems. However, the effect of carbapenems on CRE was beyond all doubt. Fourth, genotypic analysis of drug resistance genes was not performed in the study, and longer observation is needed for further analysis. Therefore, multicenter studies with a large sample size are necessary in future to address these limitations to further confirm the relationship between CRE and antibiotic usage.

Conclusion

In conclusion, β-lactamase inhibitor mixtures had different drug resistance inducing effects on CRKP and CREC. The findings will be useful for identifying specific

antimicrobial agents as targets for interventions. Rational use of antibiotics and infection control measures are urgently needed to reduce the selective pressure of antibiotics, delay the occurrence of CRE, and control its cross-transmission.

Authors' Contributions

Y.L. and J.-Y.W. designed the study. D-Q.-W. and C.W. collected the data. Y.L. and Q.X. performed the data analysis. J.C. and Y.L. wrote the article. All authors have read and critically revised the article.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Figure S1
Supplementary Figure S2

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