Original Article

Risk factors for carbapenem-resistant *Klebsiella pneumoniae* infection/colonization and predictors of mortality: a retrospective study

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Objective: To identify risk factors associated with carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infection/colonization and death and to investigate the resistance and homology of CRKP.
Methods: A retrospective 1:1 case–control study was conducted at Changhai Hospital, China, from January 2010 to December 2011.The study population included 30 patients with CRKP infection/colonization and 30 matched patients with carbapenem-susceptible *K. pneumoniae* (CSKP) infection/colonization at the same site. Homology analysis was conducted by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). Potential resistance genes were detected by PCR.
Results: Independent risk factors for CRKP infection/colonization were admission to exposure to glycopeptides [Odds ratio (OR): 43.84, 95% confidence interval (CI): 1.73–1111.91, P=0.020], cefoperazone plus sulbactam (OR: 49.56, 95% CI: 1.42–1726.72, P=0.030) and tracheostomy (OR: 677.82, 95% CI: 2.76–1667, P=0.020). Age (OR: 1.07, 95% CI: 1.00–1.14, P=0.04), renal dysfunction (OR: 17.63, 95% CI: 2.34–132.87, P=0.005) and exposure to cefoperazone plus sulbactam (OR: 8.87, 95% CI: 1.29–61.07, P=0.026) were independent risk factors for the death of patients with *K. pneumoniae* infection/colonization. Older age (OR: 1.16, 95% CI: 1.01–1.39, P=0.011) was an independent risk factor for the death of patients with CRKP infection/colonization. Thirty CRKP strains were all KPC-2-producing resistant strains with genotype of ST-11.
Conclusion: Exposure to glycopeptides, cefoperazone plus sulbactam and tracheostomy were independent risk factors for CRKP infection/colonization, and older age was an independent risk factor for CRKP infection/colonization caused death.

Keywords: Carbapenem-resistant *Klebsiella pneumoniae*, Mortality, Risk factors, Antimicrobial resistance

Introduction

Klebsiella pneumoniae (K. pneumoniae) is one of the pathogens responsible for hospital-acquired infections including pneumonia, septicaemia, urinary tract and lower biliary tract infections and hepatic abscess, especially in immunocompromised people.[1–3] Carbapenems are often used to treat infection/colonization caused by *K. pneumoniae* producing extended-spectrum beta-lactamases (ESBLs).[4] However, the increasing prevalence of antibiotic-resistant bacteria, including carbapenem-resistant *K. pneumoniae* (CRKP), has become a potentially pathogenic and fatal factor.[5] Mortality of the patients infected with CRKP seems to be higher compared to the patients infected with carbapenem-susceptible *K. pneumoniae* (CSKP).[6]

Carbapenem-resistant *K. pneumoniae* was first reported in 1997 and very rare in the world at that time.[7] A novel carbapenem-hydrolyzing beta-lactamase named KPC-1 was isolated from a carbapenem-resistant strain of *K. pneumoniae* in 2001.[8] Currently, the epidemic outbreaks of KPC-producing strains have been reported in many countries and regions.[9–11] *K. pneumoniae* is commonly present in intensive care

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unit (ICU) as a species of Enterobacteriaceae, which is most prone to producing the enzyme associated with carbapenem resistance.[12] As treatment regimens suitable for CRKP infection/colonization are limited, epidemiological and microbiological studies on carbapenem-resistant Gramme-negative bacteria appear to be particularly important.

Identifying risk factors for the development of CRKP acquisition is important for treatment options, and consequently, a number of risk factors for CRKP infection/colonization have been revealed in some retrospective clinical studies.[4,12–14] However, the conclusions were not always consistent among these studies. Antibiotic exposure was considered an independent factor for CRKP infection/colonization in some studies,[12] but not in all.[6,13] Other independent factors were prior ICU stay, renal disease, diabetes mellitus, solid tumour, tracheostomy, endoscopy, urinary catheter insertion and antipseudomonal penicillin use.[4,6,12–15]

In order to improve empirical therapy efficacy, we identified risk factors correlated with CRKP infection/colonization and mortality caused by *K. pneumoniae*. In addition, microbiological features of the strains were also detected.

Methods

Subjects and study design

This retrospective study was performed at Shanghai Changhai Hospital of China, a tertiary-care teaching hospital with 2000 beds. Patients with hospital-acquired *K. pneumoniae* infection/colonization (positive *K. pneumoniae* culture 48 hours after admission) from January 2010 to December 2011 were included in this study.

Patients inclusion criteria were hospital stay ≥ 7 days; age >18 years; no prior *K. pneumoniae* infection/colonization before the current admission; with the same site of infection/colonization for CRKP and CSKP. Carbapenem-susceptible *K. pneumoniae* - infected patients were with a difference in age of ± 5 years and *K. pneumoniae* culture time of ± 3 days compared with CRKP-infected patients. If several potential patients of the CSKP group met the qualification, only one was randomly selected.

Data collection

Data collection was performed by reviewing medical and microbiological data and recording: clinical departments where strains were isolated, patients age, gender, comorbidities such as lung disease, cardiac insufficiency, renal insufficiency, diabetes mellitus and hypoalbuminaemia, treatments before obtaining the positive culture such as mechanical ventilation, invasive procedures (deep venous catheterization, indwelling gastric tube, indwelling urethral catheter and tracheostomy), hospitalization (length of ICU staying, APCHE II score), antibiotic administration 3 months prior to the current hospitalization and during the current hospitalization until the positive culture for *K. pneumoniae* was obtained such as fluoroquinolones, first-generation cephalosporins, second-generation cephalosporins, third-generation cephalosporins, cefoperazone plus sulbactam, carbapenems (imipenem or meropenem), amikacin, glycopeptides (vancomycin, teicoplanin) and the clinical outcomes. Among them, hypoproteinemia was defined as plasma albumin <30 g/l; lung diseases included chronic obstructive pulmonary disease, chronic bronchitis, and respiratory failure; inclusion criteria for cardiac dysfunction were brain natriuretic factor or peptide (BNP) >400 pg/ml or previously conformed cardiac dysfunction. All antimicrobial agents included in the statistics were those used for at least 48 hours before *K. pneumoniae* infection/colonization.

Statistical analysis

All statistical analyses were performed using statistical software SPSS 18.0 (SPSS Inc., Chicago, IL, USA). All data were expressed as mean $\pm$ standard deviation for continuous variables or percent for categorical variables. In analysis of risk factors for CRKP infection/colonization and mortality, univariate logistic regression analysis was performed. To identify the independent risk factors, variables with $P<0.05$ in the univariate analysis were included in multivariate logistic regression model and analyzed using backward stepwise regression. Odds ratio (OR) and 95% confidence interval (CI) were also calculated. For all statistical analyses, $P<0.05$ indicated statistical significance.

Resistance mechanism and homology analysis of CRKP

Imipenem susceptibility of the strains was determined by metallo-beta-lactamases (MBLs) Etest.[16] The Hodge test was used when resistance to one of the carbapenems was demonstrated.[17] The minimum inhibitory concentration (MIC) ≥ 16 μg/ml was used as the standard of CRKP strains.[18,19]

The strain genotyping was performed using multilocus sequence typing (MLST) method. Seven housekeeping genes (rpoB, gapA, mdh, pgi, pho E, infB and tonB) were, respectively, amplified. The products were sequenced and aligned to obtain ST genotyping of the strains at http://www.Pasteur.fr/cgi-bin/genopole/PF8/mlstdbnet.pl?page=allseq&file=klebs_profiles.xml. The potential resistance genes (NDM-1, CTX and KPC) were obtained by PCR amplification. The genomic DNA of the *K. pneumoniae* strains was digested using XbaI restriction endonuclease. The obtained fragments were separated using

pulsed-field gel electrophoresis (PFGE). Pulsed-field gel electrophoresis was performed with a contour-clamped homogeneous electric field DRII apparatus from Bio-Rad Laboratories (Richmond, CA, USA). The chromosomal DNA was digested overnight with *Xba* I (Takara, Dalian, China). DNA was electrophoresed in 1.2% agarose at 6 V/cm for 24 hours; the pulse time was increased from 5 to 40 seconds. The results were analyzed using the software of Bio-Rad Quantity one. The PFGE types were analyzed according to the criteria defined by Tenover *et al.*[20]

Results

Thirty CRKP strains were separately derived from emergency department (nine strains), department of burn injury (six strains), osteology (three strains), neurology (one strain), neurosurgery (three strains), thoracic surgery (three strains), general surgery (three strains), department of gastroenterology (one strain) and pneumology (one strain). The specimens were collected from sputum (17 strains), wound secretions (6 strains), urine (3 strains), blood (2 strains), and drainage fluid (2 strains). The specimen collection departments and sites in CSKP group were in one-to-one correspondence with those in CRKP group.

Risk factors for CRKP infection/colonization: a clinical case–control study

Several factors were found to be associated with CRKP infection/colonization, including prior exposure to cefoperazone plus sulbactam, carbapenems or glycopeptide, deep venous catheterization, tracheostomy, indwelling urethral catheter, indwelling gastric tube, higher APCHE II score, ICU stay, and longer ICU length of stay ($P<0.05$) (Table 1). Among them, prior exposure to glycopeptide (OR: 43.84, 95% CI: 1.73–1111.91, $P=0.02$) or cefoperazone plus sulbactam administration (OR: 49.56, 95% CI: 1.42–1726.72, $P=0.03$), deep venous catheterization (OR: 46.88, 95% CI: 0.52–4238.09, $P=0.09$) and tracheotomy (OR: 677.82, 95% CI: 2.76–1667, $P=0.020$) were independent risk factors for CRKP infection/colonization (Table 1).

Clinical cohort study on mortality: patients who survived versus those died from K. pneumoniae infection/colonization

Among the enrolled 60 patients with *K. pneumoniae* infection or colonization, 15 (25%) patients died. Univariate analysis indicated that older age, lung

Table 1 Analysis of risk factors for patients infected with CRKP

	Variables		Univariate analysis		Multivariate analysis	
Risk factors	CRKP ($n=30$)	CSKP ($n=30$)	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age (years)	59·8 ± 17·5	60·6 ± 17·2	0·86 (0·97–1·03)	0·856		
Male, *n* (%)	25 (83·3)	5 (76·7)	0·66 (0·18–2·4)	0·52		
Concomitant diseases, *n* (%)						
Lung disease	15 (50·0)	10 (33·3)	2·0 (0·71–5·67)	0·193		
Cardiac dysfunction	8 (26·7)	8 (26·7)	1·00 (0·32–3·14)	1		
Renal dysfunction	12 (40·0)	7 (23·3)	2·19 (0·72–6·69)	0·169		
Diabetes	5 (16·7)	4 (13·3)	1·30 (0·31–5·40)	0·718		
Hypoalbuminaemia	18 (60)	13 (43·3)	1·96 (0·70–5·48)	0·199		
Tumour	5 (16·7)	11 (36·7)	0·35 (0·10–1·16)	0·086		
Antibiotics application before *Klebsiella pneumoniae* infection, *n* (%)						
Fluoroquinolone	19 (63·3)	12 (40)	2·6 (0·91–7·30)	0·073		
2nd generation cephalosporins	9 (30)	12 (40)	0·64 (0·22–1·87)	0·418		
3rd generation cephalosporins	7 (23·3)	7 (23·3)	1·00 (0·30–3·30)	1		
Amikacin	6 (20)	1 (3·3)	7·30 (0·82–64·5)	0·076		
Carbapenem	20 (66·7)	7 (23·3)	6·57 (2·10–20·48)	0·001	4·62 (0·25–84·05)	0·3
Cefoperazone plus sulbactam	15 (50)	5 (16·7)	5·0 (1·51–16·60)	0·008	49·56 (1·42–1726·72)	0·03
Tazocin	4 (13·3)	5 (16·7)	0·77 (0·19–3·19)	0·718		
Glycopeptides	17 (56·7)	4 (13·3)	8·5 (2·37–30·46)	0·001	43·84 (1·73–1111·91)	0·02
Invasive procedures, *n* (%)						
Deep venous catheterization	27 (90)	17 (56·7)	6·88 (1·71–27·8)	0·007	46·88 (0·52–4238·09)	0·09
Tracheotomy	26 (86·7)	13 (43·3)	8·5 (2·37–30·46)	0·001	677·82 (2·76–1667)	0·02
Indwelling urethral catheter	26 (86·7)	19 (63·3)	3·76 (1·04–13·64)	0·044	0·11 (0·01–4·96)	0·26
Indwelling gastric tube	25 (83·3)	16 (53·3)	4·37 (1·32–14·50)	0·016	0·05 (0·01–2·32)	0·13
APCHE2 score	22·9 ± 8·1	17·2 ± 7·4	1·10 (1·02-1·18)	0·01	1·01 (0·83-1·23)	0·92
Hospital stay (days)	33·8 ± 30·8	18·0 ± 23·4	1·03 (1·00–1·06)	0·054		
ICU stay (days)	25 (83·3)	18 (60)	3·33 (0·99–11·14)	0·048	0·54 (0·02–14·66)	0·72
Hospital stay before bacteraemia (days)	33·8 ± 30·8	13·2 ± 27·4	1·03 (1·01–1·06)	0·02	1·09 (0·98–1·2)	0·1
Outcomes						
Death	10 (33·3)	5 (16·7)				
Improvement	20 (66·7)	25 (83·3)				

CRKP: carbapenem-resistant *Klebsiella pneumonia*; CSKP: carbapenem-sensitive *Klebsiella*; OR: odds ratio; CI: confidence interval; ICU: intensive care unit.The variables screened in univariate analysis with $P<0·05$ were included in the multivariate logistic regression analysis after repeated verification of the fitted model.

diseases, cardiac dysfunction, renal dysfunction, exposure to cefoperazone plus sulbactam and longer ICU length of stay were associated with the death of the *K. pneumoniae*-infected/colonized patients ($P<0.05$) (Table 2). Multivariate analysis showed that older age (OR: 1.07, 95% CI: 1.00–1.14, $P=0.04$), renal dysfunction (OR: 17.63, 95% CI: 2.34–132.87, $P=0.005$) and exposure to cefoperazone plus sulbactam (OR: 8.87, 95% CI: 1.29–61.07, $P=0.026$) were independent risk factors associated with *K. pneumonia* infection/colonization induced death (Table 2).

Clinical cohort study on mortality: patients who survived versus those died from CRKP infection/colonization

Ten of the 30 patients with CRKP infection/colonization died. Univariate analysis indicated that the factors such as older age, renal dysfunction and exposure to cefoperazone plus sulbactam were associated with death ($P<0.05$) (Table 3). Among them, only older age (OR: 1.16, 95% CI: 1.01–1.39, $P=0.011$) was the independent risk factor for death of patients with CRKP infection/colonization (Table 3).

Resistance mechanism and homology analysis of CRKP

All of the 30 imipenem-resistant CRKP strains (MIC $\geq$ 16 μg/ml) were multiresistant (susceptibility to polymyxin was not measured). The main resistance mechanism of the 30 CRKP strains was attributed to KPC production. The genotypes of all strains were ST-11 (Fig. 1).

Discussion

Since the reports on CRKP, treating *K. pneumoniae* infection/colonization using carbapenems has been challenged. To improve empirical therapy efficacy, we studied the risk factors for CRKP infection/colonization. Our investigation indicates that CRKP infection/colonization was associated with various factors such as ICU stay, prior antibiotic use and invasive procedures. Exposure to glycopeptides or cefoperazone plus sulbactam as well as tracheotomy were independent risk factors for CRKP infection/colonization; age, renal insufficiency and cefoperazone plus sulbactam administration were independent risk factors for death of *K. pneumoniae*-infected patients; older age was an independent risk factor for death of patients with CRKP infection/colonization. These findings suggested that the clinicians should place emphasis on the appropriate antibiotic use and aseptic invasive procedures.

Our study suggested that ICU staying and longer length of ICU stay before *K. pneumoniae* infection/colonization were associated with CRKP infection/

Table 2 Analysis of risk factors associated with *Klebsiella pneumoniae* infection/colonization induced death

	Variables		Univariate analysis		Multivariate analysis	
Risk factors	Death ($n=15$)	Survival ($n=45$)	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age (years)	71·3 ± 13·8	56·5 ± 16·8	1·07 (1·02–1·12)	0·007	1·07 (1·00–1·14)	0·04
Male, *n* (%)	11 (73·3)	37 (82·2)	0·59 (0·15–2·35)	0·459		
Concomitant diseases, *n* (%)						
Lung disease	10 (66·7)	15 (33·3)	4 (1·16–13·82)	0·028		
Cardiac dysfunction	7 (46·7)	9 (20)	3·5 (1–12·22)	0·049	1·05 (0·16–6·7)	0·96
Renal dysfunction	10 (66·7)	9 (20)	8 (2·18–29·31)	0·002	17·63 (2·34–132·87)	0·005
Liver dysfunction	1 (6·7)	1 (2·2)	3·14 (0·18–53·59)	0·429		
Diabetes	2 (13·3)	7 (15·6)	0·84 (0·15–4·54)	0·835		
Hypoalbuminaemia	9 (60)	22 (48·9)	1·57 (0·48–5·14)	0·458		
Tumour	4 (26·7)	12 (26·7)	1 (0·27–3·75)	1		
Antibiotics application before *K. pneumoniae* infection, *n* (%)						
Fluoroquinolone	11 (73·3)	20 (44·4)	3·44 (0·95–12·45)	0·06		
2nd generation cephalosporins	4 (26·7)	17 (37·8)	0·6 (0·16–2·18)	0·437		
3rd generation cephalosporins	6 (40)	8 (17·8)	3·08 (0·85–11·14)	0·086		
Amikacin	2 (13·3)	5 (11·1)	1·23 (0·21–7·12)	0·817		
Carbapenem	9 (60)	18 (40)	2·25 (0·68–7·42)	0·183		
Cefoperazone plus sulbactam	9 (60)	11 (24·4)	4·64 (1·35–15·97)	0·015	8·87 (1·29–61·07)	0·026
Tazocin	3 (20)	6 (13·3)	1·62 (0·35–7·5)	0·534		
Glycopeptides	5 (33·3)	16 (35·6)	0·91 (0·26–3·12)	0·876		
Invasive procedures, *n* (%)						
Deep venous catheterization	13 (86·7)	31 (68·9)	2·94 (0·58–14·79)	0·192		
Tracheotomy	12 (80)	27 (60)	2·67 (0·66–10·8)	0·69		
Indwelling urethral catheter	12 (80)	33 (73·3)	1·45 (0·35–6·06)	0·607		
Indwelling gastric tube	12 (80)	29 (64·4)	2·21 (0·54–8·99)	0·269		
Surgery	4 (26·7)	24 (53·3)	0·32 (0·09–1·15)	0·081		
APCHE2 score	30·7 ± 4·3	16·5 ± 5·7	10·67 (0·87–130·64)	0·064		
ICU stay (days)	13 (86·7)	30 (66·7)	3·25 (0·65–16·3)	0·152		
Hospital stay before bacteraemia, days	41 ± 41·2	18 ± 24·5	1·02 (1·00–1·04)	0·031	1·01 (0·98–1·03)	0·48

OR: odds ratio; CI: confidence interval; ICU: intensive care unit.The variables screened in univariate analysis with $P<0·05$ were included in the multivariate logistic regression analysis after repeated verification of the fitted model.

Table 3 Analysis of risk factors associated with CRKP infection/colonization induced death

	Variables		Univariate analysis		Multivariate analysis	
Risk factors	**Death (n=10)**	**Survival (n=20)**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
Age (years)	73·6 ± 11·1	52·9 ± 16·0	1·11 (1·03–1·20)	0·009	1·16 (1·01–1·39)	0·011
Male, *n* (%)	7 (70)	18 (90)	0·26 (0·04–1·9)	0·18		
Concomitant diseases, *n* (%)						
Renal dysfunction	7 (70)	5 (25)	7 (1·29–37·91)	0·024	185·69 (0·91–37 797·14)	0·054
Antibiotics application before *K. pneumoniae* infection, *n* (%)						
Cefoperazone plus sulbactam	8 (80)	7 (35)	7·43 (1·23–45·01)	0·029	319·05 (0·68–149 157·23)	0·07

CRKP: carbapenem-resistant *Klebsiella pneumonia*; OR: odds ratio; CI: confidence interval; ICU: intensive care unit.The variables screened in univariate analysis with $P<0·05$ were included in the multivariate logistic regression analysis after repeated verification of the fitted model.

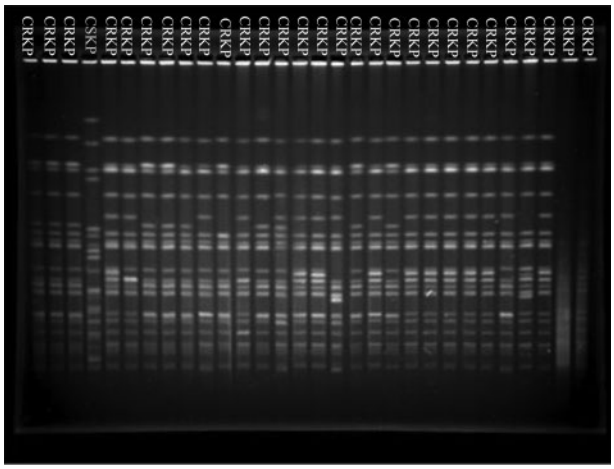

Figure 1 Pulsed-field gel electrophoresis (PFGE) patterns of carbapenem-resistant *Klebsiella pneumoniae* (CRKP). The fourth lane indicated ESBL + CSKP (ATCC 700603), and the others indicated CRKP strains.

colonization, which was consistent with the studies conducted by Gregory *et al.*[21] and Schwaber *et al.*[22] Most patients admitted to the ICU have relatively serious complications. Furthermore, the airborne and contact transmission of resistant bacteria in the ICU environment may lead to nosocomial infection/colonization. The patients with prolonged ICU stay may undergo more invasive procedures and may be treated with a longer duration of antibiotics use or with broad spectrum antibiotics. All the above factors can lead to the emergence of carbapenems-resistant bacteria.

Carbapenems administration has been proven to be an independent risk factor for carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infection/colonization.[23,24] Our study suggested that carbapenems administration prior to *K. pneumoniae* infection was also associated with CRKP infection, which was consistent with many previous retrospective clinical studies.[22,25–29] The fluoroquinolones or quinolones utilization was also an independent risk factor for CRKP infection/colonization[29] and death.[26,30] Laboratory evidences indicated that plasmid-encoded quinolone resistance determinant gene causing low-level fluoroquinolone resistance was located on *K. pneumoniae* plasmids encoding KPC gene (especially blaKPC-2 and qnrB4). However, the present study as well as the studies conducted by Gregory *et al.*,[21] Kwalk *et al.*[25] and Wu *et al.*[31] failed to prove the correlation between fluoroquinolones use and CRKP infection/colonization. This might be due to the fact that quinolones abuse in some regions had resulted in non-significant difference in the clinical studies.

We also found that glycopeptide or cefoperazone plus sulbactam administration were risk factors for CRKP infection/colonization, which was consistent with the previous finding.[31] Long-term application of glycopeptides would significantly inhibit the growth of Gramme-positive bacteria and thus lead to more frequent mutations and drug resistance of Gramme-negative bacteria. However, cefoperazone plus sulbactam administration could not be explained by this possible mechanism. Further microbiological researches remain to be performed.

Our studies suggested that tracheotomy was an independent risk factor associated with CRKP infection/colonization. Frequent invasive operations and tube indwelling may cause respiratory tract or gastrointestinal mucosal injury easily. The mucosal barrier injury will cause further decline in the body resistance and increase in probabilities of bacterial colonization or infection/colonization.

Besides, this study concluded that older age, concomitant disease (renal insufficiency) and application of cefoperazone plus sulbactam were independent risk factors for death caused by *K. pneumoniae* infection/colonization. Advanced age, severe underlining diseases and inadequate antibiotic treatment would reduce the immunity, which increased the risk of infection/colonization and even death.

Most of the present clinical studies focus on the risk factors for acquisition of CRKP, but few reports

combined the mechanisms of resistance. The risk factors and sensitivity of antibiotic agent of CRKP may not differ with different mechanisms of resistance, so microbiological detection of *K. pneumoniae* was particularly included in our study. Analysis on the genotypes and homogeneity of the strains suggested that KPC-2 was the main factor causing antibiotic resistance in CRKP strains. Some researchers also indicated that the resistance mechanisms of 53% CRKP strains were due to KPC-2 producing.[24,32–34] In addition to producing KPC enzyme, the CRKP resistance mechanisms also included the loss of outer membrane proteins and production of beta-lactam-hydrolyzing enzymes.[35]

Our study also has some limitations. First, we should acknowledge that the sample size was not large enough, which might lead to errors in statistical analysis and the omission of some other risk factors. Second, there was not an established criterion for differentiating infection from colonization of *K. pneumoniae* in the study. In addition, this study was only conducted in one tertiary-care teaching hospital. Prospective, multicenter clinical trials are expected to be performed.

Conclusion

This retrospective study indicated that exposure to glycopeptides or cefoperazone plus sulbactam, as well as tracheostomy, were independent risk factors for severe infection/colonization caused by CRKP. These findings may provide some recommendation for the diagnosis and treatment of patients infected with KPC-producing CRKP strains in Shanghai, China.

Disclaimer Statements

Contributors All listed authors participated meaningfully in the study and have seen and approved the final manuscript.

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Conflicts of interest We declare that we have no conflicts of interest.

Ethics approval This retrospective study was performed in Shanghai Changhai Hospital of China.

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