Epidemiology and clinical significance of pLVPK-like virulence plasmid in KPC-2-producing Klebsiella pneumoniae infections in eastern China: a preliminary exploration

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Research

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Abstract

Background: By acquiring a pLVPK-like virulence plasmid (pVir), *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* (KPC-kp) evolves to a hypervirulent variant, with increasing cases reported worldwide. However, little is known about the epidemiological trend of pVir in KPC-kp, as well as clinical characteristics of infections caused by this novel strain (pVir$^+$-KPC-kp).

Methods: From 2014-2018, 662 carbapenem-resistant *K. pneumoniae* (CRKP) were consecutively collected from a tertiary hospital of eastern China. The confirmed KPC-kp were subjected to antimicrobial susceptibility testing, multilocus sequence typing and detection of pLVPK-related genetic loci (*rmpA*, *rmpA2*, *iucA* and *iroN*) to identify pVir$^+$-KPC-kp strains. Then, the clinical characteristics and outcomes of KPC-kp infection patients with and without pVir were compared. Moreover, the risk factors for pVir$^+$-KPC-kp infections also determined by a multivariable logistic regression analysis.

Results: of the 662 CRKP, 86.6% (573/662) were KPC-kp including 285 (49.7%) pVir$^+$-KPC-kp and 288 (50.3%) pVir$^-$-KPC-kp. Notably, the prevalence of pVir$^+$-KPC-kp by year increased remarkably from 2014 (19.5%, 8/41) to 2018 (60.0%, 90/150). Sequence type (ST) 11 was the most predominant ST, accounting for 88.9% of all pVir$^+$-KPC-kp. For the 352 KPC-kp infection cases (186 with pVir$^+$-KPC-kp and 166 with pVir$^-$-KPC-kp), multivariable analysis indicated neurosurgery (Odds ratio [OR], 2.92; 95% confidence interval [CI], 1.48-5.75; *P* =0.002) was independently associated with pVir$^+$-KPC-kp infections. Although patients with pVir$^+$-KPC-kp infections had higher incidence of septic shock (31.2% vs. 21.1%, *P* =0.03), the two groups showed no significant differences in 7-day mortality (23.1% vs. 18.1%, *P* =0.24) or 28-day mortality (45.7% vs. 44.0%, *P* =0.75).

Conclusions: Altogether, wide dissemination of pVir in ST11 KPC-kp has emerged in China. Neurosurgery is an independent risk factor for acquisition of pVir$^+$-KPC-kp infections. The mortality rates were similar between patients infected with pVir$^+$-KPC-kp or pVir$^-$-KPC-kp, suggesting uncertain impact of pVir in clinical outcome.

Background

During the last decades, a new variant called hypervirulent *Klebsiella pneumoniae* (hvKP) causing severe invasive infections in apparently healthy people has posed a particular threat to public health [1, 2]. pLVPK originates from *K. pneumoniae* CG43 strain [3]. Virulence plasmid carrying pLVPK-borne genes termed as pLVPK-like plasmid (pVir) is commonly detected in hvKP clones, such as sequence type (ST) 23 [4]. A correlation has been established between pVir and high-level virulence [5]. Some virulence genes harbored by pVir, including *rmpA*/*rmpA2* (regulating capsular polysaccharide biosynthesis), *iucABCD-iutA* (encoding aerobactin siderophore) and *iroBCDN* (encoding salmochelin siderophore), are proved to be better biomarkers for hvKP than the widely used “string test” [1, 6].

Contrary to hvKP, carbapenem-resistant *K. pneumoniae* (CRKP) is recognized as an important pathogen associated with nosocomial infections in immunocompromised patients [7]. Since first reported in China in 2007 [8], *Klebsiella pneumoniae* carbapenemase (KPC) has become the most predominant contributor to carbapenem resistance in CRKP of China [9]. It is a common sense that KPC-producing *K. pneumoniae* (KPC-kp) is absent of pVir and nearly avirulent experimentally [10]. However, convergent *K. pneumoniae* strains of widely distributed ST11 carrying both pVir and KPC ($pVir^+$-KPC-kp) have emerged, especially in China [11-13]. So far, most cases of $pVir^+$-KPC-kp infections were still isolated or sporadic. However, recently, new evidence alerted that a rapidly increasing prevalence of $pVir^+$-KPC-kp might occur due to the potential spread of a novel conjugative IncFIB plasmid containing bla_{KPC} and key virulence-encoding region of pVir simultaneously [14, 15]. Therefore, there is an urgent need to evaluate the current epidemiological trend of pVir in KPC-kp.

Besides displaying high levels of resistance to commonly used antibiotics, $pVir^+$-KPC-kp strains in general exhibited enhanced anti-phagocytic and serum resistance properties *in vitro* and were associated with extremely high mortality both in affected patients and experimental models [11, 12]. However, the association of virulence traits and pVir in multidrug-resistant *K. pneumoniae* remains controversial and the clinical features of patients infected with $pVir^+$-KPC-kp strains are less revealed [16, 17].

To address these problems, in present study, we first investigated the prevalence of pVir among KPC-kp collected from a tertiary hospital in eastern China over four consecutive years. Then, we characterized the clinical features and outcomes of $pVir^+$-KPC-kp infections by comparing with $pVir^-$-KPC-kp strains. Also, the risk factors of $pVir^+$-KPC-kp infections were determined.

Methods

Design, Setting and Ethics

This retrospective cohort study was performed in The First Affiliated Hospital, College of Medicine, Zhejiang University, from July 2014 to August 2018. It is one of the largest hospitals in eastern China, having approximate 2500 beds and 131,000 annual admissions. The first report of KPC-kp in China came from here in 2007 [8]. Since then, KPC-kp has been endemic in the hospital [18].

The present study was conducted according to the principles stated in the Declaration of Helsinki and approved by the ethical review board of The First Affiliated Hospital, College of Medicine, Zhejiang University.

Patient Cohort

The source population consisted of all patients admitted to the hospital during the study period, whose bacterial cultures were identified as CRKP. The clinical specimens mainly included blood, sputum/tracheal aspirate, pleural fluid and ascites. Further detailed collection of clinical information was performed for the patients whose isolates were verified as KPC-kp. Patients aged <18 years and those

with missing or incomplete clinical records were excluded. Patients with polymicrobial infections and those with KPC-kp colonization were excluded as well. Infection or colonization was defined according to the criteria of the Center for Disease Control and Prevention of America [19]. Only the first episode for each patient was included in our analysis.

Clinical Data Collection

For all eligible KPC-kp infection cases, chart review was performed to obtain the clinical data. Infectious disease specialists independently collected the following clinical information using the patients' electronic charts and/or paper records: demographic conditions, unit and duration of hospitalization, underlying diseases, Charlson Comorbidity Index, abscess information and previous invasive procedures. Patients were defined as immunosuppressed if they had HIV or AIDS, were post-transplant, had received chemotherapy within 4 weeks, had received immunosuppressive agents for more than 2 weeks, or had neutropenia. Previous use of antibiotics was defined as antimicrobial therapy (intravenous or oral) within 4 weeks before infections. Invasive procedures included surgery and mechanical ventilation performed within 4 weeks prior to the infections. Appropriate empirical or definitive antimicrobial therapy was defined as previously described [18].

Outcome Variables

The primary outcome variables were all-cause mortality at day 7 and day 28. Other secondary outcome variables were infection-related mortality and in-hospital mortality. Septic shock and multiple organ dysfunction syndrome (MODS) at the onset of infections, as well as the length of stay (LOS) from culture to discharge, were also assessed.

Bacterial Identification and Antimicrobial Susceptibility Testing

Bacterial isolates identification and antimicrobial susceptibility testing were performed using Vitek 2 automated system (bioMérieux, France) and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) 2019 criteria [20]. For tigecycline, colistin and ceftazidime-avibactam (CAZ/AVI), minimum inhibitory concentrations (MICs) were determined using broth microdilution method. The susceptibility results were categorized in accordance with the CLSI criteria for CAZ/AVI, while European Committee on Antimicrobial Susceptibility Testing (version 9.0, http://www.eucast.org/clinical_breakpoints/) for colistin and tigecycline. The species identification was verified by MALDI-TOF MS (Bruker Daltonics Inc., Fremont, CA, USA). *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC 25922 were used as the quality control strains of antimicrobial susceptibility testing.

Molecular Analysis

All of the CRKP were subjected to detection of bla_{KPC} by PCR. The isolates carrying bla_{KPC} were further investigated for pLVPK-related genetic loci *rmpA*, *rmpA2*, *iucA* and *iroN*. The primers used for PCR

amplification and sequencing were acquired from a previous study [11]. Considering the primers of *rmpA* and *rmpA2* target only the plasmid-borne genes, while *iucA* and *iroN* for both plasmid-borne and chromosomally-encoded genes, KPC-kp isolates of positive *rmpA* or *rmpA2* were defined as pVir^{+}-KPC-kp according to the definition previously described [11, 21-23].

Multilocus sequence typing (MLST) was carried out according to the protocol described on the *K. pneumoniae* MLST website ((https://bigsdb.pasteur.fr/klebsiella/klebsiella.html), and the results were analysed using the corresponding database.

Statistical Analysis

Data were analysed using SPSS software (version 20; SPSS Inc., Chicago, IL, USA). Categorical variables were presented as absolute numbers and their relative frequencies, and analyzed by chi-square or Fisher exact test. For continuous variables, the Kolmogorov-Smirnov test was applied to distinguish between normal and non-normal distribution. Normally distributed data were expressed as mean and standard deviation (SD) and analysed using Student's t-test, while non-normally distributed data were present with median and interquartile rang (IQR) and compared using Mann-Whitney test. To identify independent predictors for pVir^{+}-KPC-kp infections, we used logistic regression. All variables with *P* value <0.1 in univariate analysis as well as clinical importance were incorporated into multivariable regression model. *P* value <0.05 was considered statistically significant.

Results

During the study period, a total of 662 CRKP isolates were collected. Of these, 573 were KPC-kp, and further were analysed for antimicrobial testing and pLVPK-derived loci. Two hundred and eighty-five isolates were identified as pVir^{+}-KPC-kp and 288 as pVir^{-}-KPC-kp. There were 352 cases met the criteria and included in the final analysis of clinical characteristics. The causes of exclusion are shown in Figure 1, mainly polymicrobial infections (104 cases) and colonization (86 cases).

Prevalence of pVir in KPC-kp

The proportion of pVir among KPC-kp isolates increased continuously and significantly between 2014 and 2018 (19.5% in 2014, 18.5% in 2015, 49.5% in 2016, 77.5% in 2017 and 60.0% in 2018, respectively). Among the 285 pVir^{+}-KPC-kp isolates, pVir (*rmpA*$^{+}$ *rmpA2*$^{+}$ *iucA*$^{+}$ *iroN*$^{+}$) and pVir (*rmpA2*$^{+}$ *iucA*$^{+}$) were the two most predominant virulence plasmid types, accounting for 55.1% (157/285) and 28.4% (81/285) respectively. The annual distribution of different types of pVir was shown in Figure 2 and Table S1.

Among the 285 pVir^{+}-KPC-kp, 90 isolates were randomly selected for MLST analysis. Overall, these strains were assigned to seven different STs, with ST11 the most common type (88.9%, 80/90), followed by ST23 (3.3%, 3/90) and ST1660 (2.2%, 2/90). Additionally, ST65, ST152, ST660, ST893 and ST268 were found in one isolate each.

Antimicrobial resistance

Susceptibility testing showed significantly higher resistance rates of aminoglycosides, cefepime and trimethoprim-sulfamethoxazole in pVir$^+$-KPC-kp than pVir$^-$-KPC-kp (*P* <0.001, =0.02 and <0.001, respectively). However, the CZA/AVI resistance rate of pVir$^-$-KPC-kp was 4.9%, which was much higher than that of pVir$^+$-KPC-kp (4.9% vs. 0.35%, *P* =0.001). Although the results did not reach statistical significance, the resistance rates of pVir$^+$-KPC-kp against tigecycline and colistin were higher than those of pVir$^-$-KPC-kp (Figure 3, Table S2).

Predictors and Survival Impact of pVir$^+$-KPC-kp Infections

The clinical characteristics of the cohort are described in Table 1. Among the 352 episodes of KPC-kp infections, 186 (52.8%) were due to pVir$^+$-KPC-kp and 166 (47.2%) were due to pVir$^-$-KPC-kp. The age, sex, hospital ward and abscess formation did not differ between these two groups. The source of infections and prior antibiotic exposure except cephalosporins (pVir$^+$-KPC-kp 7.0% vs. pVir$^-$-KPC-kp 2.4%, *P* =0.045) in both groups were similar as well. pVir$^-$-KPC-kp infections were more associated with immunosuppression state (35.5% vs. 25.3%, *P* =0.04). In contrast, a significantly more patients with pVir$^+$-KPC-kp infections had Charlson comorbidity index < 3 (36.6% vs. 23.5%, *P* =0.008). The pVir$^+$-KPC-kp infections were also more associated with underlying cerebral vascular disease (25.8% vs. 15.7%, *P* =0.02) and performance of neurosurgery within 4 weeks prior to infections (22.0% vs. 7.8%, *P* <0.001) (Table 1). However, only neurosurgery remained associated with pVir$^+$-KPC-kp infections in multivariable analysis (OR, 2.92; 95% CI, 1.48-5.75; *P* =0.002) (Table 2).

Unexpectedly, although patients infected with pVir$^+$-KPC-kp had higher incidence of septic shock (31.2% vs. 21.1%, *p* = 0.03), the two groups did not differ significantly with respect to 7-day, 28-day, overall and infected-related mortality (Table 3).

Furthermore, multivariate analysis of 130 patients with bloodstream infections and 126 intensive care unit (ICU) patients also indicated neurosurgery was the only independent predictor for pVir$^+$-KPC-kp infections. Besides, among these patients, those infected with pVir$^+$-KPC-kp and pVir$^-$-KPC-kp had similar clinical outcomes as well (data not shown).

Discussion

The present study found a high prevalence and increasing trend of pVir in ST11 KPC-kp in our hospital during the past four years. We identified previous neurosurgery within 4 weeks as the only clinical factor independently associated with pVir$^+$-KPC-kp infections. Although septic shock was more frequently occurred in patients with pVir$^+$-KPC-kp, the two groups did not differ significantly in terms of mortality. Most recently, a multicenter molecular epidemiological study of carbapenem-resistant hypervirulent *K.*

pneumoniae (CR-hvKP) in China was reported, while limited clinical data was available [24]. As our best knowledge, it is so far the largest retrospective cohort study of KPC-kp with and without pVir.

Firstly, we screened the carriage of pVir as previously described [11]. Consistent with previous reports, most pVir$^+$-KPC-kp belonged to the epidemic ST11 [23, 25, 26]. Notably, the prevalence of pVir in KPC-kp in 2014 had already reached 19.5%, much higher than that reported from Gu et al (3% in 2015) [11], suggesting an earlier transmission timeline in our hospital. Most recently, the research of Yang et al indicated a 100kb fragment of pVir integrated into a transmissible *bla*$_{KPC}$-bearing IncFIB plasmid, which would prompt the dissemination of pVir among CRKP [14]. Based on the facts that the detection of pVir$^+$-KPC-kp increased from 2014 to 2018 and ST11 pVir$^+$-KPC-kp gradually becomes to be the predominant clone, it is speculated a transmission of IncFIB plasmid carrying both pVir-fragment and *bla*$_{KPC}$ among ST11 KPC-kp might have happened in our region. Studies aiming to test this hypothesis would be very imperative. Furthermore, we found nearly half of the pVir$^+$-KPC-kp (44.9%) were absent of one or more pVir-featured genes, suggesting common deletion events in the process of plasmid fragment integration.

As most studies focused on the molecular epidemiological investigation of pVir$^+$-KPC-kp [24, 25], little was known about the risk factors of infections caused by this newly emerged variant. Here, we showed that previous neurosurgery within 4 weeks could help clinicians to identify patients of high risk to acquire pVir$^+$-KPC-kp infections (p = 0.002). Our previous study reported that about half of *K. pneumoniae* meningitis was caused by pVir$^+$-KPC-kp in our hospital from 2011 to 2017 [27]. In order to establish the correlation of neurosurgery and meningitis, we performed subgroup analysis of post-neurosurgery patients. However, it is of note that, most of the post-neurosurgical patients (46.3%, 25/54) were presence of pVir$^+$-KPC-kp pneumonia and 35.1% developed to bacterial meningitis. There was no statistical significance of detection sites of pVir$^+$-KPC-kp between post-neurosurgical patients and other patients. Moreover, for the patients of bloodstream infection and patients in ICU, neurosurgery within 4 weeks was also the independent factors of pVir$^+$-KPC-kp infections. Further prospective survey and molecular epidemiology will clarify the correlation between neurosurgery and pVir$^+$-KPC-kp infection, as well as the mode of transmission.

In *K. pneumoniae*, the terms “hypervirulence” primarily referred to isolates displaying hypermucoviscosity and causing invasive infections. However, most recent studies viewed the pVir$^+$-KPC-kp strains associated with an extremely high infectious mortality as CR-hvKP, even if there was a lack of clinical features due to hvKP infections [11, 13]. In present study, we compared some well-recognized parameters that could assist in differentiating infection due to hypervirulent and classical *K. pneumoniae* strains (such as infection development location, patients' age and abscess formation) [1], there was no difference between two groups. Moreover, our results indicated the 7-day, 28-day, overall and infection-related mortality were similar between patients with pVir$^+$-KPC-kp and pVir$^-$-KPC-kp infections. Although the inconsistencies in patient care or variability in types of infections might introduce bias to the analysis, further evaluation of the association between pVir$^+$-KPC-kp infections and mortality for ICU patients and patients with BSIs drew the same conclusion. Furthermore, we also noted 11.6% patients (33/285)

isolated with pVir^{+}-KPC-kp were absent of any symptoms of infections, who eventually did not develop to invasive infections and had favorable outcomes. Therefore, it was reasonable to believe not all of the KPC-kp would evolve to hypervirulent variants and resulted in fatalities by acquiring pVir. Study on the comprehensive genome analysis of ST11 pVir^{+}-KPC-kp strains with varied virulence level is underway to provide more clues on the virulence mechanisms of this newly emerged strains.

There are some important limitations of our current study that should be acknowledged. First, the study was a single-center study with KPC-kp infected patients enrolled retrospectively. Our hospital located in Zhejiang province in China, where ST11 KPC-kp was epidemic [18] and a fatal outbreak caused by ST11 CR-hvKP was reported here in 2017 [11], therefore, the generalization of our results should be approached with caution. Second, the virulence of serial pVir^{+}-KPC-kp strains that associated with variable prognosis had not be evaluated experimentally, and we just took the clinical mortality as the virulence measure. However, the present study firstly compared the clinical outcomes between patients infected with pVir^{+}-KPC-kp and pVir^{-}-KPC-kp.

Conclusions

Altogether, we observed a high prevalence of pVir in KPC-kp and ST11 KPC-kp with pVir as the predominant clone in our region. Moreover, we also demonstrated the clinical features and mortality was similar between infections caused by KPC-kp with and without pVir. Our finding indicated that not all of the KPC-kp could evolve to hypervirulent variant by acquiring pVir, therefore, the notion that KPC-kp harboring pVir was equivalent to hypervirulent strain should be strongly considered. Regardless, in order to prevent the dissemination of *K pneumoniae* strain with hyper-resistance and hypervirulence, active surveillance for these KPC-kp strains carrying pVir alone with virulent evaluation was still necessary. Additionally, research into exploration of virulence mechanism of pVir^{+}-KPC-kp strains may help to treat these infections.

Abbreviations

pVir: pLVPK-like virulence plasmid, KPC: *Klebsiella pneumoniae* carbapenemases, KPC-kp: (KPC)-producing *K. pneumoniae*, CRKP, Carbapenem-resistant *K. pneumoniae*, ST: Sequence type, OR: Odds ratio, CI: Confidence interval, hvKP: Hypervirulent *Klebsiella pneumoniae*, MODS: Multiple organ dysfunction syndrome, LOS: Length of stay, CLSI: Clinical and Laboratory Standards Institute, CAZ/AVI: Ceftazidime-avibactam, MICs: Minimum inhibitory concentrations, MLST ; Multilocus sequence typing, SD: Standard deviation, ICU, intensive care unit, CR-hvKP: Carbapenem-resistant hypervirulent *K. pneumoniae*.

Declarations

Ethics approval and consent to participate

Ethical approval was granted from the Ethics Committees and review board of the First Affiliated Hospital, College of Medicine, Zhejiang University. As this study used secondary data, informed consent was not obtained from patients.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MX and YF designed the study. MX and QY performed data analysis and drafted the manuscript. YL performed the genotypic analysis of the clinical isolates. HK and ZF participated in the collection of strains and clinical information. XC performed the antimicrobial susceptibility testing. YC and YF reviewed the paper and provided recommendations. All authors read and approved the final manuscript.

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Tables

Table 1. Clinical characteristics of 352 patients with KPC-kp infections divided into groups by presence of pVir.

Variable	All isolates (n=352)	pVir^{+}-KPC-kp (n=186)	pVir^{-}-KPC-kp (n=166)	*P*-value
Demographics data				
Age, years, (mean ±SD)	59.1±16.3	57.9±15.7	60.4±16.8	0.15
Male gender	248 (70.5)	130 (69.9)	118 (71.1)	0.81
Charlson comorbidity index <3	107 (30.4)	68 (36.6)	39 (23.5)	**0.008**
Ward submitting index culture				
ICU	126 (35.8)	66 (35.5)	60 (36.1)	0.9
Medical	169 (48.0)	86 (46.2)	83 (50.0)	0.48
Surgical	55 (15.6)	34 (18.3)	21 (12.7)	0.15
Underlying diseases				
Hypertension	132 (37.5)	70 (37.6)	62 (37.3)	0.96
Diabetes mellitus	74 (21.0)	35 (18.8)	39 (23.5)	0.29
Hematological malignancy	23 (6.5)	9 (4.8)	14 (8.4)	0.17
Solid tumor	78 (22.2)	37 (19.9)	41 (24.7)	0.28
Cirrhosis	42 (11.9)	18 (9.7)	24 (14.5	0.17
Solid organ transplantation	60 (17.0)	27 (14.5)	33 (19.9)	0.18
Chronic kidney disease	40 (11.4)	16 (8.6)	24 (14.5)	0.08
Chronic lung disease	36 (10.2)	22 (11.8)	14 (8.4)	0.29
Cardiovascular disease	63 (17.9)	33 (17.7)	30 (18.1)	0.94
Trauma	49 (13.9)	30 (16.1)	19 (11.4)	0.21
Cerebral vascular disease	74 (21.0)	48 (25.8)	26 (15.7)	**0.02**
Abscess formation				
Liver abscess	3 (0.9)	1 (0.5)	2 (1.2)	0.60
Non-hepatic abscess	28 (8.0)	16 (8.6)	12 (7.2)	0.64
Non-abscess	321 (91.2)	169 (90.9)	152 (91.6)	0.82
Invasive procedures				
Surgery before infection	177 (50.3)	102 (54.8)	75 (45.2)	0.07
Neurosurgery	54 (15.3)	41 (22.0)	13 (7.8)	**<0.001**

Mechanical ventilation	162 (46.3)	91 (48.9)	71 (43.3)	0.29
Detection site				
Blood stream	130 (36.9)	64 (34.4)	66 (39.8)	0.3
Low respiratory tract	112 (31.8)	62 (33.3)	50 (30.1)	0.52
Intra-abdominal	58 (16.5)	28 (15.1)	30 (18.1)	0.45
Cerebrospinal fluid	23 (6.5)	13 (7.0)	10 (6.0)	0.72
Urogenital	20 (5.7)	13 (7.0)	7 (4.2)	0.26
Skin and soft tissue	9 (2.6)	6 (3.2)	2 (1.8)	0.51
Prior antibiotic exposure				
Cephalosporins	17 (4.8)	13 (7.0)	4 (2.4)	**0.045**
β-lactam and β-lactamase inhibitors	112 (31.8)	55 (29.6)	57 (34.3)	0.34
Carbapenems	142 (40.3)	73 (39.2)	69 (41.6)	0.66
Aminoglycosides	2 (0.6)	1 (0.5)	1 (0.6)	1.00
Fluoroquinolones	10 (2.8)	5 (2.7)	5 (3.0)	0.86
Tigecycline	52 (14.8)	31 (16.7)	21 (12.7)	0.29
Others	16 (4.5)	7 (3.8)	9 (5.4)	0.46
Immunosuppression state	106 (30.1)	47 (25.3)	59 (35.5)	**0.04**

Data are presented as No. (%) unless otherwise specified;

ICU, intensive care unit.

Table 2. Univariable and multivariable analysis of risk factors for pVir+-KPC-kp infections.

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Charlson comorbidity index <3	1.56	1.12-2.17	**0.008**			
Surgery before infection	1.21	0.98-1.50	0.07			
Neurosurgery within 4 weeks prior to infections	2.82	1.56-5.07	**<0.001**	2.92	1.48-5.75	**0.002**
Immunosuppression state	0.71	0.52-0.98	0.04			
Chronic kidney disease	0.60	0.33-1.08	0.08			
Cerebral vascular disease	1.65	1.07-2.53	**0.02**			
Liver abscess	0.45	0.04-4.88	0.60			
Non-hepatic abscess	1.19	0.58-2.44	0.64			
Previous exposure to cephalosporins	2.90	0.97-8.72	**0.045**			

OR, odds ratio; CI, confidence interval.

Table 3. Clinical outcomes of patients infected with pVir+-KPC-kp and pVir–KPC-kp.

Variable	All isolates (n=352)	pVir^{+}-KPC-kp (n=186)	pVir^{-}-KPC-kp (n=166)	*P*-value
Appropriate empirical antimicrobial therapy	58 (16.5)	37 (19.9)	21 (12.7)	0.07
Appropriate definite antimicrobial therapy	205 (58.2)	116 (62.4)	89 (53.6)	0.10
MODS	142 (40.3)	69 (37.1)	73 (44.0)	0.28
Septic shock	93 (26.4)	58 (31.2)	35 (21.1)	**0.03**
LOS from culture to discharge, days, median (IQR)	17 (7-120)	16 (6.8-120)	19 (8-120)	0.42
Mortality				
7-day mortality	73 (20.7)	43 (23.1)	30 (18.1)	0.24
28-day mortality	158 (44.9)	85 (45.7)	73 (44.0)	0.75
Infection-related mortality	131 (37.2)	74 (39.8)	57 (34.3)	0.29
In-hospital mortality	191 (54.3)	107 (57.5)	84 (50.6)	0.19

Data are presented as No. (%) unless otherwise specified;

MODS, multiple organ dysfunction syndrome; LOS, length of stay; IQR, interquartile range.

Figures

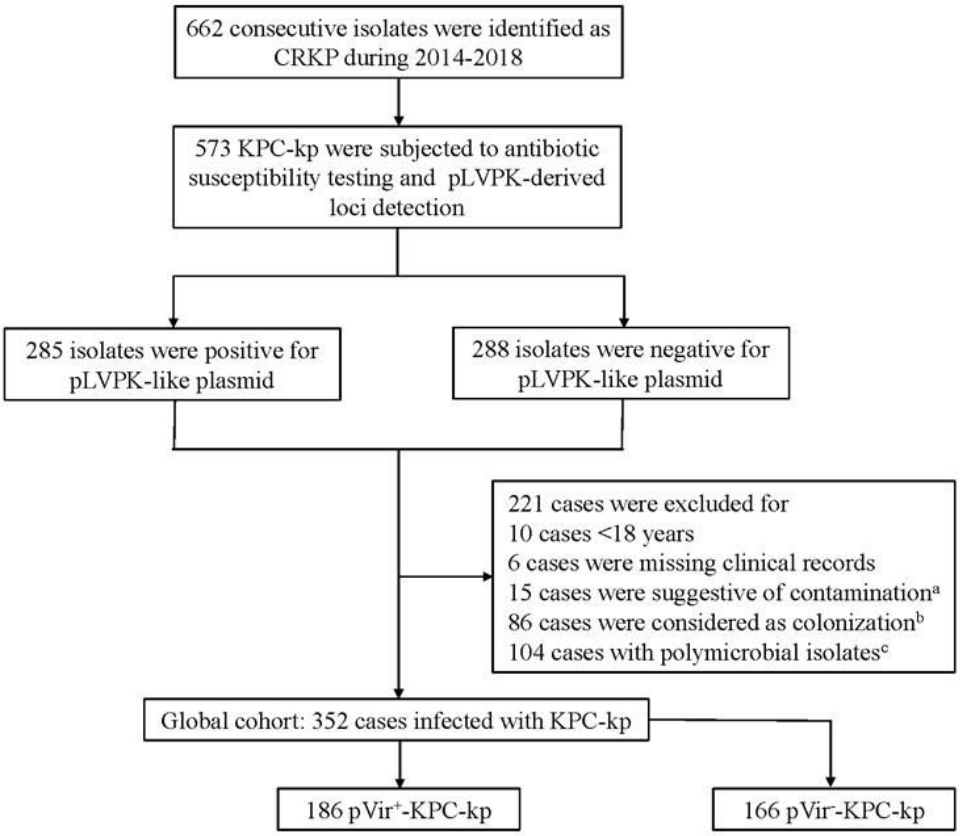

Figure 1

Flowchart for selection of 352 patients with KPC-kp infections. acontamination is defined as the presence of K. pneumonia in sterile fluid, without clinical signs or symptoms suggestive of infection; bcolonization is defines as the presence of K. pneumonia in a sputum, stool or urine specimen, in the absence of systemic signs of inflammatory response; clncluding 41 case with Acinetobacter baumannii, 22 case with Pseudomonas aeruginosa, 11 case with Enterococcus faecium, 8 case with Stenotrophomonas maltophilia, 5 case with Escherichia coli, 3 case with Staphylococcus aureus, 3 case with Elizabethkingia meningoseptica, 2 case with Serratia marcescens, 2 case with Enterobacter cloacae, 1 case with Morganella morganii, 1 case with Burkholderia cepacia, 1 case with Enterococcus faecalis, 1 with Moraxella catarrhalis, 1 case with Cryptococcus neoformans, 1 case with Candida tropicalis and 1 case with Candida glabrata.

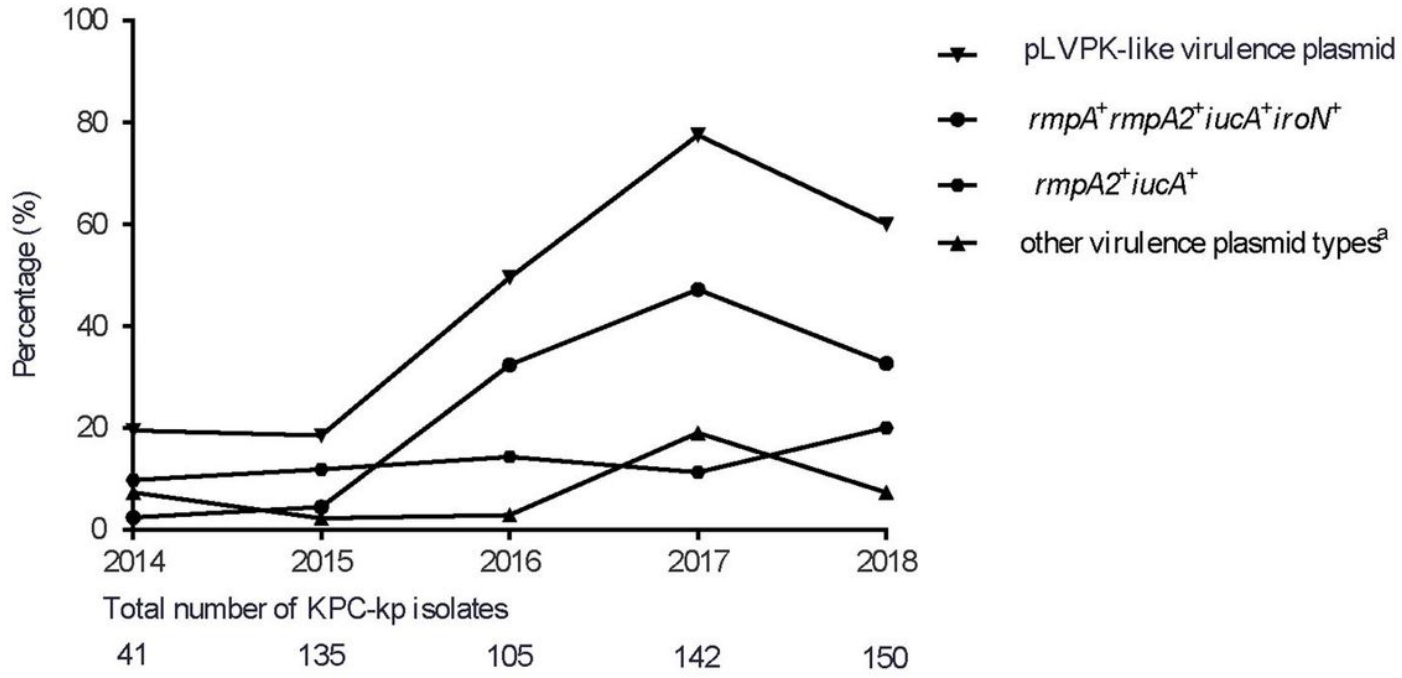

Figure 2

Annual distribution of the pVir types. a listed in Table S1.

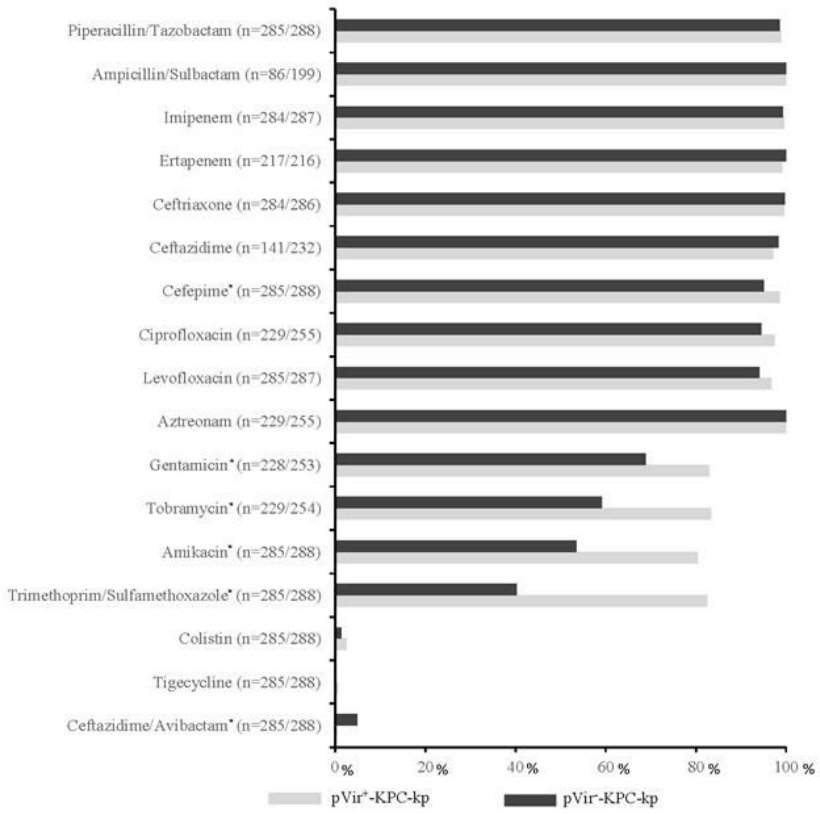

Figure 3

A comparison of the antibiotic susceptibility testing between pVir+-KPC-kp and pVir–KPC-kp strains. *P <0.05