Molecular Epidemiology, Risk Factors and Outcomes of Carbapenem-Resistant *Klebsiella Pneumoniae* Infection in a Tertiary Hospital of Eastern China: For a Retrospective Study Over 4 Years

Dongmei Zhao

First affiliated hospital of Anhui medical University

Hongru Li

First affiliated hospital of Anhui medical University

Chengcheng Yue

First affiliated hospital of Anhui medical University

Yanyan Liu

First Affiliated Hospital of Anhui Medical University

Ying Ye

First Affiliated Hospital of Anhui Medical University

Yabin Li (✉ lijabin@ahmu.edu.cn)

First Affiliated Hospital of Anhui Medical University <https://orcid.org/0000-0003-3238-569X>

Research

Keywords: Carbapenem-resistant *Klebsiella pneumoniae*, Epidemiology, ST11, Risk factors, Outcomes

Posted Date: November 8th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-71433/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) have undergone extensive dissemination in worldwide resulting in increased mortality. We performed a retrospective analysis of epidemiology and risk factors for the outcome of CRKP infection in a general teaching hospital in China.

Methods

A molecular and clinical study were conducted for 98 CRKP in a tertiary hospital from January 2013 to December 2016. Carbapenemase gene detection, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were performed. Logistic regression was also used to identify the risk factors associating with the 30-day mortality.

Results

The production of KPC carbapenemase was the main resistant mechanism and increased annually with a significant difference. However, the molecular outcome revealed the dominance and diversity in CRKP with 24 sequence types (STs) and 59 PFGE types (PTs). The ST11 CRKP were documented as the predominant strains in our study, which showed a significant increasing trend year by year. Additionally, the predominant ST11 CRKP corresponding to PT10 and PT15 remained a typical fashion. Of note, the new advantage PT09 or PT16 were just discovered in 2016 which also corresponding to ST11. Meanwhile, the factors on 30-day mortality and ST11 proportionality with CRKP infection were assessed, which showed that ST11, appropriate empirical treatment, and hospital stays were independently associated with 30-day mortality.

Conclusions

ST11 CRKP played a dominant role in the process, but the homology of these strains was polymorphic and the advantage clusters would be changed by evolution. Additionally, besides appropriate empirical treatment and hospital stays, ST11 CRKP was independently associated with the 30-day mortality, first reported as we know.

Background

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has emerged as a major nosocomial pathogen and are being increasingly reported worldwide [1–3], which has been associated with a high mortality rate range from approximately 16–70% [4, 5]. Carbapenem resistance in *K. pneumoniae* is reported to be caused either by epidemic clones or by the horizontal dissemination of mobile elements. Additionally, the

predominant production of *Klebsiella pneumoniae* carbapenemase (KPC) contributed to the most important mechanism of carbapenem resistance in *K. pneumoniae* [6]. Of epidemiological significance, the international spread of KPC-producing *K. pneumoniae* is primarily associated with a single multilocus sequence type (ST) or its related variants. In Europe and America, KPC-producing ST258 *K. pneumoniae* is regarded as one of the most successful multidrug-resistant nosocomial pathogens [3]. However, the international high-risk clone of *K. pneumoniae* ST11 is frequently reported as a successful pathogen at infections in Asia [6–8]. Of note, Wang et al [9] reported that KPC-positive ST11 *K. pneumoniae* with a highly resistant to meropenem had a significant difference on the mortality.

Indeed, data regarding the distribution of these resistant determinants among CRKP isolates collected during a longtime period is still limited in China, and the relationship between molecular characteristics and the clinical outcome is also sparing. In our study, we aimed to describe the epidemiology of CRKP, focus on the molecular characteristics of circulating strains and to evaluate which proportion of strains was susceptible to the clinical outcome.

Methods

1. Study population

A retrospective study for hospitalized specimens which were isolated with CRKP bacteria from January 2013 to December 2016 was conducted in a hospital with 2800 beds. 327 strains of CRKP bacteria (no repeated isolates) were screened out, including 98 strains of nosocomial CRKP pathogenic bacteria. Medical charts were reviewed and demographic and clinical information was collected. This study was approved by the Ethics Committee of the first affiliated hospital of Anhui Medical University.

2. Bacterial isolates and genotypic investigation of resistance

Non-duplicate *K. pneumoniae* isolates which developed 48 h following hospital admission were collected. These isolates were subsequently characterized as hospital-acquired infections with resistant to imipenem, meropenem or ertapenem. Identification and antimicrobial drug susceptibility testing were performed using automated systems from VITEK®2 (bioMérieux, France). Organisms were considered resistant to carbapenems according to the CLSI guidelines (2016) [10]. Polymerase chain reaction (PCR) for the common *bla*_{KPC} gene was carried out in order to identify the carbapenemase gene of all CRKP.

3. Molecular typing

To track and characterize the clonal relationships of the *K. pneumoniae* isolates, pulsed-field gel electrophoresis (PFGE) of the XbaI-digested genomic DNA was conducted using a CHEF-DRIII system (Bio-Rad, United Kingdom). Strain relatedness analysis was performed on the BioNumerics software version based on the Dice coefficient (SD) for genetic relatedness. SD values reflect the degree of similarity between PFGE images of different strains, ranging from 0 to 1, where 0 represents completely different and 1 represents completely identical. Different bands are judged to be different types.

According to the similarity coefficient between each two images, the unweighted pair group average method (UPGMA) was used for clustering to construct the cluster tree, which is described as the reference [11]. Concomitantly, multi locus sequence typing (MLST) was the most common technique, which based on genetic variation in seven housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB* and *tonB*) that together provided a relative genetic profile. Then, CRKP isolates were assigned a ST number according to the allelic profiles available in the Institute Pasteur database. These ST data can be further defined by eBURST.

4. Clinical characterization and definitions

Clinical data was collected using electronic medical records which extract patient information, including demographic characteristics, chronic underlying diseases, invasive procedures, disease status, antimicrobial therapy, clinical outcomes with 30-day mortality. 1). Infection: all patients were evaluated according to CDC criteria [12] to assess whether the infection was due to CRKP. 2). Emergency sate was defined as life-threatening organ dysfunction, which was evaluated by an acute change in total Sequential Organ Failure Assessment (SOFA) score of ≥ 2 [13]. 3). Empirical and definitive therapy: treatment administered before the susceptibility testing results were available was characterized as empirical, whereas treatment given after the results were available (at least one active drug for ≥ 48 h) was considered as definitive therapy. The former is divided into appropriate treatment and inappropriate treatment in our study. Appropriate antibiotic treatment was defined as appropriate antimicrobial therapy with at least one active agent initiated within 48 h after infection onset[14]. Definitive treatment regimens were classified as monotherapy (treatment with one in vitro active agent) and combination therapy (treatment with two or more in vitro active agents), and the latter included associated with Tigecycline or with others. 4). Clinical outcome: Infection-related mortality was defined as the proportion of patients who died only as a direct consequence of the CRKP infection, and with no other plausible explanation in the opinion of the local investigator. Therefore, 30-day infection-related mortality was considered as death for patients who died within 30 days of the onset of the CRKP infection.

5. Statistical analysis

Statistical analysis was performed using SPSS version 21 software (IBM Corp., Armonk, NY). Univariate analysis was performed to identify factors related to mortality and the ST11 of CRKP. The chi-square test was used for categorical variables and Student's t-test for continuous variables. A logistic regression model was used to identify factors independently associated with 30-day-mortality. Sets of variables that had $P \leq 0.1$ in the univariate analysis on mortality were entered into the model. P-values were interpreted together with 95% confidence interval (CI) for the logistic regression model. All tests were two tailed and a P-value of < 0.05 was considered statistically significant.

Results

1. Isolates

A total of 98 patients with CRKP infection were identified during the study period. Of these, the median age was 62 years, with nearly three-quarters were male, and 42 patients (42.9%) were admission to intensive care unit (ICU). The 98 non-duplicate CRKP isolates were obtained from sputum samples (71/98, 72.4%), blood cultures (13/98, 13.3%) and other samples (14/98, 14.3%), the main sites of infection are the lungs and blood system. Based on antibiotic resistance testing for these isolates, imipenem MICs ranged from 2 mg/L to 16 mg/L (two intermediate isolates), or meropenem MICs ranged from 4 mg/L to 16 mg/L, or ertapenem MICs present 2 mg/L (imipenem and/or meropenem MIC $\geq$ 4 mg/L, ertapenem MIC $\geq$ 2 mg/L). Antimicrobial alternatives to carbapenems demonstrating through automated systems included the resistant rates of 100% to ampicillin/sulbactam, more than 93% to aztreonam, ciprofloxacin and piperacillin/tazobactam. Amikacin, gentamicin and levofloxacin showed resistant rates of 80.6%, 82.7% and 88.78%, respectively. And all isolates except two intermediate ones were susceptible to Tigecycline. Of these isolates, 60 (60/98, 61.2%) yielded a clinical specimen with the predominant *bla*_{KPC} positive CRKP and 55 isolates (55/98, 56.1%) were classified into ST11. During the four periods across the year of 2013 to 2016, we found that CRKP infection had no statistical significance in the distribution of age, gender, ICU admission and sample source, however, the CRKP isolates carrying *bla*_{KPC} showed an increasing trend year by year, and which showed significantly different between the three periods ($p < 0.001$). Accordingly, a significantly higher prevalence of isolates containing ST11 CRKP ($p < 0.001$) was also noted in the near year (Table 1).

2. Characterization of CRKP isolates by Molecular analysis

In this study, 24 different STs were identified in these 98 *K. pneumoniae* isolates by MLST analysis, the most dominant sequence type was ST11 (56.1%, 55/98), followed by ST23 (11.2%, 11/98), ST15 and ST86 (3.1%, 3/98 respectively). And each of the 6 STs (ST17/ST147/ST340/ST307/ST268/ST65) belonged to 2 isolates, while the remaining 14 STs obtained 1 strain, respectively. Among these, 10 STs of strains produced KPC β -lactamase, of which the predominant ST11 harbored 49 KPC-producing *K. pneumoniae* isolates (89.1%, 49/55) and ST23 contained 4 KPC-positive ones (36.4%, 4/11), the remaining STs possessed only one, respectively.

Of the 98 isolates, 3 isolates were ruled out for the degradation of chromosome nucleic acid. Thus, 95 were available for PFGE, and the analysis revealed 59 different clonal PFGE types (PTs, PT01-PT59). Of those, the highest percentage clone was named PT15 (15.8%, 15/95), followed by PT10 (8.4%, 8/95), PT06 and PT11 (4.2%, 4/95, respectively), PT13 (3.2%, 3/95), and PT01, PT04, PT07, PT08, PT09, PT16 and PT36 (2.1%, 2/95, respectively), the remaining PTs possessed only one, respectively. Interestingly, ST11 also occupied predominant isolates according to the prepotent PFGE pattern. 54 CRKP isolates (the actual was 55 strains for one nucleic acid degradation) of 23 PTs clones corresponded to the same type of ST11, the top type clones were recommended as PT15 (25.9%, 14/54), PT10 (13.0%, 7/54), PT06 and PT11 (7.4%, 4/54, respectively). According to clinical departments, total of 16 STs were observed in ICU admission, the ST11 (57.1%, 24/42) and ST23 (7.1%, 3/42) commanded the foremost position, harboring 25 (59.5%, 25/42) KPC-2-positive *K. pneumoniae* isolates. As well, the dominant clone PT10 and PT15 corresponded to the advantage type of ST11 in ICU. It is worthy noting that the only two strains of CRKP

with clone type of PT09 or PT16 were just discovered from ICU admission in 2016 which corresponding to ST11 (Figure 1).

3. Outcome and ST11

During the study period, 87 patients with CRKP infection were included in the analysis of clinical outcome, while the remaining ones were excluded due to incomplete electronic information or for the inability to follow up with the study. With available outcome data, 32 patients (36.8%, 32/87) died within 30 days of the onset of the CRKP infection, while 55 patients (63.2%, 55/87) did not die within the time. To identify potential risk factors for 30-day mortality, infected patients who died due to CRKP infection were compared with the survived patients (Table 2). The 30-day mortality was mainly male (71.9%, 23/87) with a mean±standard deviation age 60.19±21.31 years. On univariate analysis, no significant difference in sex, age, specimen source, underlying disease, emergency state, invasive operation, combination with other strain and definitive therapy were observed. However, the length of hospitalization was associated with 30-day mortality with a certain significance ($p = 0.088$), as well as the admission to ICU ($p = 0.061$). Of note, the 30-day mortality of patients treated with appropriate empirical treatment was 9.4% (3/32) and the survivor was 45.5% (25/55), which showed a significant difference ($p < 0.001$) (Table 2).

Due to the tendency of ST11 CRKP in hospital mentioned above, we analyzed the possible risk factors depending on clinical data. ST11 CRKP and non-ST11 CRKP were compared in Table 2. The patients with ST11 CRKP infection had a significant emergency state than those with non-ST11 ($p = 0.03$). Additionally, the operative treatment and appropriate empirical treatment both showed a borderline significance with the ST11 CRKP infection ($p = 0.075$ or $p = 0.056$, respectively). Then, no significant statistical difference in other related variables was found between the ST11 CRKP and non-ST11 strains.

A logistic regression analysis was conducted in order to investigate the association between the 30-day mortality and risk factors, including the length of hospitalization, ICU admission, appropriate empirical treatment, emergency state and ST11 CRKP, which unlike the outcome that the univariate analysis is considered. The results revealed that the length of hospitalization (odds ratio [OR], 0.961, 95% confidence interval [CI] 0.935-0.988, $P = 0.004$), appropriate empirical treatment (OR, 11.301, 95% CI, 2.47-51.71, $P = 0.002$) and ST11 CRKP (OR, 0.193, 95% CI, 0.056-0.669, $P = 0.01$) were factors independently associated with 30-day mortality. Admission to ICU and emergency state were not found with significant statistical difference (Table 3).

Discussion

Nosocomial infections due to CRKP are associated with substantial mortality, yet the clinical significance of isolating CRKP from the hospital is unknown. To our knowledge, this study is the first to describe the epidemiology and 30-day mortality of patients with CRKP infection from the long-term retrospective observation. Through this extensive inquiry, a clear picture has emerged which gradually reveal the particularly noteworthy findings. First of all, due to the trend of the KPC-positive CRKP strains belonging

to the dominant ST11 lineage were observed, thus, our investigation took further insight on the epidemiology. The outcome stressed the diversity in CRKP strains with 24 clones of ST and 59 clusters of PT, however, the strains of predominant ST11 CRKP corresponding to PT10 and PT15 remained in vogue. Importantly, it is worthy noting that the new advantage cluster of PT09 or PT16 were just discovered from ICU admission in 2016 which corresponding to ST11. Secondly, the 30-day mortality of the patients due to CRKP infection was associated with the pathogens of ST11 CRKP. Summary together, our findings provided a new insight into the risk factors of the clinical outcome and further demonstrated the importance of epidemiology.

In our study, the investigation was conducted on CRKP isolates in 2013-2016. The molecular epidemiological methods had been used for tracking and characterization of CRKP isolates by PFGE and MLST which were accepted to differentiate the species—both of which indicated that CRKP ST11 was identified as the predominant sequence type strain in our study. The epidemiology of CRKP is continually evolving, and now the advantage strains of *bla*_{KPC}-producing *K. pneumoniae* ST11 have been reported, which is the most frequently detected KPC-producing *K. pneumoniae* clone in China [15–17]. Interestingly, ST11 also occupied predominant isolates according to the prepotent PFGE pattern. As well, the dominant clone PT10 and PT15 corresponded to the advantage type of ST11 in ICU where took a important role in acquisition of carbapenem resistant isolates [18]. It is worthy noting that the only two strains of CRKP with clone type of PT09 or PT16, only second to the dominant cluster PT, were just discovered from ICU admission in 2016 which corresponding to ST11. In this study, it stated that ST11 CRKP payed a dominant role in the process, but aslo emphasized that the homology of these strains was polymorphic and the advantage clusters were changed by evolution. Naturally, an efficient measure could be established to monitor the variation of the prevalence of these CRKP strains.

In this report, the overall 30-day mortality rate of patients infected with CRKP was 36.78% (32/87), which was similar to that reported by previous studies for CRKP infections [19, 20]. Several previous studies [21–23] had showed that the long-term hospitalization and empirical treatment were independent predictors of death. Through our retrospective study, the length of hospitalization and appropriate empirical treatment both were independently associated with 30-day mortality. The key point of this study was to evaluate the correlation of ST11 CRKP infection with the clinical outcomes, which was first reported, as we know so far [7–9].

Next, this focused investigation on the ST11 CRKP would be taken into consideration. Sequence types of globally prevalent carbapenem-resistant *Enterobacteriaceae*, including ST11 of *K. pneumoniae* clonal complex 258, were considered as a high-risk clones [24]. Thus, we believe that the analysis of type ST11 and the association with clinical baseline factor were concerning. Though the underlying mechanisms behind the emergence of CRKP ST11 clones, as well as their associations with the outcomes are yet to be elucidated, we used our acquisition of knowledge by reviewing the relevant literature and attempted to provide some perspectives on the issue. Closely related to ST258, ST11's factors that contribute to the epidemiologic success remain unknown. However, several articles report that chromosomal or plasmid factors, beyond antibiotic resistance, may increase the strain's fitness and provide an advantage that

underlies its prevalence [25, 26]. Additionally, a recent report [27] showed that the KPC-producing *K. pneumoniae* ST11 clone was resistant to serum killing, which may also be proof for the prevalence. In addition, a review [28] explained that ST11 genome strains carried distinct capsular polysaccharide (cps) regions, and the cps locus was one of the primary determinants of antigenicity associated with *K. pneumoniae*. Capsule switching was a species-specific mechanism used by the microbe to escape the host immune response. Croucher, *et al* [29] inferred that DNA exchange in-and-around the cps regions may be an important mechanism used by *K. pneumoniae* to rapidly diversify and evolve. Therefore, the success prevalence of CRKP ST11 may be due largely to chromosomal recombination instead of antibiotic resistance. Brisse S, *et al* [30] found that strains owning some serotypes usually carried a relevant virulence factor content and had been associated with serious human infections, which underscored the potential threat represented by the emergence and diffusion of carbapenem-resistance clones with increased virulence potential [31, 32]. Meanwhile, CRKP associated with plasmid-encoded carbapenemases, which could acquire multiclass antibiotic resistance to produce distinct clinical challenges and result in invasive infections with high mortality. Undoubtedly, careful monitoring of carbapenem susceptibilities and rapid identification of epidemiological lineages was quite necessary for implementation of infection-control measures to prevent the endemicity of CRKP.

Our study has several limitations. This paper does not unify the the patients of CRKP infection, difference from the other observational studies, which were based on available data that include only patients with bacteremia or bacteriuria [33–35]. Additionally, for the fewer strains acquired from the saved isolates in 2014, the study appeared to be incomplete. And, our further research should take insight into the virulent CRKP ST11 strains. Together this research will allow us to expand our knowledge on such strains.

Conclusion

ST11 CRKP played a dominant role in the process, but the homology of these strains was polymorphic and the advantage clusters would be changed by evolution. Additionally, besides appropriate empirical treatment and hospital stays, ST11 CRKP was independently associated with the 30-day mortality. Therefore, efforts are imminently needed to improve the understanding to the knowledge of the epidemiological status to prevent its spread.

Abbreviations

CRKP, Carbapenem-resistant *Klebsiella pneumoniae*, PFGE, pulsed-field gel electrophoresis, MLST, multilocus sequence typing, KPC, *Klebsiella pneumoniae* carbapenemase, ST, sequence type, PT, PFGE type, CLSI, Clinical and Laboratory Standards Institute, PCR, Polymerase chain reaction, ICU, intensive care unit.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the first affiliated hospital of Anhui Medical University (Quick- PJ 2017-11-19) and consent to participate.

Consent for publication

All authors have seen and approved for publication.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No.81673242)

Authors' contributions

Prof. JB L contributed substance to ideas and design, DM Z and HR L contributed equally to this work, drafted the paper or responsible for the experiment, CC Y and YY L collected some clinical material, Y Y offered help in test operation. All authors have read and approved the final version of the manuscript.

Acknowledgements

Not applicable.

References

1. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. *J Infect Dis* 2017,215:S28-S36.
2. Tängdén T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment and infection control. *J Intern Med*. 2015,277:501-512.
3. Tzouveleakis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clin Microbiol Rev* 2012,25:682-707.
4. Ben-David D, Kordevani R, Keller N, Tal I, Marzel A, Gal-Mor O, et al. Outcome of carbapenem resistant *Klebsiella pneumoniae* bloodstream infections. *Clin Microbiol Infect* 2012,18:54-60.
5. Petrosillo N, Giannella M, Lewis R, Viale P. Treatment of carbapenem-resistant *Klebsiella pneumoniae*: the state of the art. *Expert Rev Anti-Infect Ther* 2013,11:159-177.

6. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *The Lancet infectious diseases*. 2013;13:785-796.
7. Qi Y, Wei Z, Ji S, Du X, Shen P, Yu Y. ST11, the dominant clone of KPC-producing *Klebsiella pneumoniae* in China. *Journal of Antimicrobial Chemotherapy*. 2010;66:307-312.
8. Andrade LN, Curiao T, Ferreira JC, Longo JM, Clímaco EC, Martinez R, et al. Dissemination of blaKPC-2 by the spread of *Klebsiella pneumoniae* clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among Enterobacteriaceae species in Brazil. *Antimicrobial agents and chemotherapy*. 2011;55:3579-3583.
9. Wang X, Wang Q, Cao B, Sun S, Zhang Y, Gu B, et al. Retrospective Observational Study from a Chinese Network of the Impact of Combination Therapy versus Monotherapy on Mortality from Carbapenem-Resistant Enterobacteriaceae Bacteremia. *Antimicrob Agents Chemother*. 2018;63. pii: e01511-e01518.
10. Wayne, PA. Clinical and Laboratory Standards Institute Performance standards for antimicrobial susceptibility testing: twenty-sixth informational supplement M100-S26. CLSI, 2016.
11. Han H, Zhou H, Li H, Gao Y, Lu Z, Hu K, et al. Optimization of pulse-field gel electrophoresis for subtyping of *Klebsiella pneumoniae*. *International journal of environmental research and public health*. 2013;10:2720-2731.
12. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *American journal of infection control*. 2008;36:309-332.
13. Seymour CW, Liu VX, Iwashyna TJ, et al. Assessment of clinical criteria for sepsis: for the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8): 762-774. doi:10.1001/jama.2016.0288.
14. Zarkotou O, Pournaras S, Tselioti P, Dragoumanos V, Pitiriga V, Ranellou K, et al. Predictors of mortality in patients with bloodstream infections caused by KPC-producing *Klebsiella pneumoniae* and impact of appropriate antimicrobial treatment. *Clinical Microbiology and Infection*. 2011;17(12):1798-1803.
15. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*: a key pathogen set for global nosocomial dominance. *Antimicrob Agents Chemother* 2015;59:5873-5884.
16. Liu J, Yu J, Chen F, Yu J, Simner P, Tamma P, Liu Y, Shen L. Emergence and establishment of KPC-2-producing ST11 *Klebsiella pneumoniae* in a general hospital in Shanghai, China. *Eur J Clin Microbiol Infect Dis* 2018;37:293-299.
17. Sun K, Chen X, Li C, Yu Z, Zhou Q, Yan Y. Clonal dissemination of multilocus sequence type 11 *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* in a Chinese teaching hospital. *Apmis*. 2015;123:123-127.
18. Katchanov J, Asar L, Klupp EM, Both A, Rothe C, König C, et al. Carbapenem-resistant Gram-negative pathogens in a German university medical center: prevalence, clinical implications and the role of

- novel β -lactam/ β -lactamase inhibitor combinations. PLoS ONE 2018,13:e0195757.
19. Giannella M, Trecarichi EM, Giacobbe DR, De Rosa FG, Bassetti M, Bartoloni A, et al. Effect of combination therapy containing a high-dose carbapenem on mortality in patients with carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection. *Int J Antimicrob Agents*. 2018,51:244-248.
 20. Chiu S-K, Chan M-C, Huang L-Y, Lin Y-T, Lin J-C, Lu P-L, et al. Tigecycline resistance among carbapenem-resistant *Klebsiella Pneumoniae*: Clinical characteristics and expression levels of efflux pump genes. *PloS one*. 2017,12:e0175140.
 21. Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, et al. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: importance of combination therapy. *Clinical Infectious Diseases*. 2012,55(7):943-950.
 22. Tumbarello M, Trecarichi EM, De Rosa FG, Giannella M, Giacobbe DR, Bassetti M, et al. Infections caused by KPC-producing *Klebsiella pneumoniae*: differences in therapy and mortality in a multicentre study. *Journal of Antimicrobial Chemotherapy*. 2015,70:2133-2143.
 23. Girometti N, Lewis RE, Giannella M, Ambretti S, Bartoletti M, Tedeschi S, et al. *Klebsiella pneumoniae* bloodstream infection: epidemiology and impact of inappropriate empirical therapy. *Medicine*. 2014,93:298-309.
 24. Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. *Clinical microbiology reviews*. 2015,28:565-591.
 25. Chmelnitsky I, Shklyar M, Hermesh O, Navon-Venezia S, Edgar R, Carmeli Y. Unique genes identified in the epidemic extremely drug-resistant KPC-producing *Klebsiella pneumoniae* sequence type 258. *Journal of antimicrobial chemotherapy*. 2012,68:74-83.
 26. Cottell JL, Saw HT, Webber MA, Piddock LJ. Functional genomics to identify the factors contributing to successful persistence and global spread of an antibiotic resistance plasmid. *BMC microbiology*. 2014,14:168.
 27. Chiang T-T, Yang Y-S, Yeh K-M, Chiu S-K, Wang N-C, Lin T-Y, et al. Quantification and comparison of virulence and characteristics of different variants of carbapenemase-producing *Klebsiella pneumoniae* clinical isolates from Taiwan and the United States. *Journal of Microbiology, Immunology and Infection*. 2016,49:83-90.
 28. Chen L, Mathema B, Chavda KD, DeLeo FR, Bonomo RA, Kreiswirth BN. Carbapenemase-producing *Klebsiella pneumoniae*: molecular and genetic decoding. *Trends in microbiology*. 2014,22:686-696.
 29. Croucher NJ, Klugman KP. The emergence of bacterial "hopeful monsters". *MBio*. 2014,5(4):e01550-14.
 30. Brisse S, Fevre C, Passet V, Issenhuth-Jeanjean S, Tournebize R, Diancourt L, et al. Virulent clones of *Klebsiella pneumoniae*: identification and evolutionary scenario based on genomic and phenotypic characterization. *PloS one*. 2009,4:e4982.
 31. Zhang Y, Zeng J, Liu W, Zhao F, Hu Z, Zhao C, et al. Emergence of a hypervirulent carbapenem-resistant *Klebsiella pneumoniae* isolate from clinical infections in China. *Journal of Infection*.

2015,71:553-560.

32. Zhang R, Lin D, Chan EW-c, Gu D, Chen G-X, Chen S. Emergence of carbapenem-resistant serotype K1 hypervirulent *Klebsiella pneumoniae* strains in China. *Antimicrobial agents and chemotherapy*. 2016,60:709-711.
33. Tofas P, Skiada A, Angelopoulou M, Sipsas N, Pavlopoulou I, Tsaousi S, et al. Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections in neutropenic patients with haematological malignancies or aplastic anaemia: Analysis of 50 cases. *International journal of antimicrobial agents*. 2016,47:335-359.
34. Daikos GL, Tsaousi S, Tzouveleki LS, Anyfantis I, Psychogiou M, Argyropoulou A, et al. Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. *Antimicrobial agents and chemotherapy*. 2014,58:2322-2328.
35. Qureshi ZA, Syed A, Clarke LG, Doi Y, Shields RK. Epidemiology and clinical outcomes of patients with carbapenem-resistant *Klebsiella pneumoniae* bacteriuria. *Antimicrob Agents Chemother*. 2014,58:3100-3104.

Tables

Table 1. Carbapenem-resistant *Klebsiella pneumoniae* case characteristics.

	Total	2013-2014	2015	2016	P value
	(n=98, %)	(n=24, %)	(n=36, %)	(n=38, %)	
Age (years) (mean $\pm$ S.D.)	61.58 $\pm$ 19.07	61.46 $\pm$ 22.16	63.49 $\pm$ 17.09	58.90 $\pm$ 19.06	0.728
Gender (male)	72 (73.5)	18 (75)	27 (75)	27 (71.1)	0.911
ICU of admission	42 (42.9)	10 (41.7)	16 (44.4)	16 (42.1)	0.971
Specimen source					0.945
Sputum	71 (72.4)	17 (70.8)	26 (72.2)	28 (73.7)	
Blood	13 (13.3)	3 (12.5)	4 (11.1)	6 (15.8)	
Others	14 (14.3)	4 (16.7)	6 (16.7)	4 (10.5)	
KPC-2	60 (61.2)	4 (16.7)	23 (63.9)	33 (86.8)	< 0.001
ST11	55 (56.1)	4 (16.7)	22 (61.1)	29 (76.3)	< 0.001

S.D., standard deviation; ICU, intensive care unit; KPC, *Klebsiella pneumoniae* carbapenemase; ST, sequence type.

Table 2. Univariate analysis of factors associated with 30-day mortality and ST11 for 87 patients with carbapenemase-resistant *Klebsiella pneumoniae* infection.

	30-day-mortality (n=32, %)	1. day-survivor (n=55, %)	<i>P</i> value	ST11 (n=53, %)	Non-ST11 (n=34, %)	<i>P</i> value
Gender, male	23 (71.9)	42 (76.4)	0.642	38 (71.7)	27 (79.4)	0.75
Age (years) (mean±S.D.)	60.19 ± 21.31	63.73 ± 15.60	0.376	60.19 ± 19.22	65.91 ± 15.16	0.146
The length of hospitalization (mean±S.D.)	32.5 ± 21.69	40.02 ± 29.35	0.088	37.87 ± 24.30	39.53 ± 31.24	0.782
ICU admission	17 (53.1)	18 (32.7)	0.061	24 (45.3)	11 (32.4)	0.23
Specimen source			0.421			0.261
Sputum	22	41		40	23	
Blood	6	5		8	3	
Others	4	9		5	8	
Underlying disease	24 (75)	42 (76.4)	0.886	39 (73.6)	27 (79.4)	0.535
Emergency state	23 (71.9)	35 (63.6)	0.432	40 (75.5)	18 (52.9)	0.03
Operative treatment	10 (31.3)	14 (25.5)	0.56	11 (20.8)	13 (38.2)	0.075
Invasive operation	5 (15.6)	15 (27.27)	0.213	14 (26.4)	6 (17.7)	0.343
Combination with other bacterium	23(71.9)	33 (60)	0.605	35 (66.0)	21 (61.8)	0.685
Appropriate empirical treatment	3 (9.4)	25 (45.45)	< 0.001	13 (25.4)	15 (44.1)	0.056
Definitive therapy			0.521			0.121
Monotherapy	6	15		12	9	
Combination with Tigecycline	19	26		24	21	
Combination with carbapenem	7	14		17	4	

Table 3. Factors associated with 30-day mortality in 87 patients with carbapenemase-resistant *Klebsiella pneumoniae* infection by Logistic regression model.

Variables	Odds ratio	95% Confidence interval		P value
		lower	upper	
The length of hospitalization	0.961	0.935	0.988	0.004
ICU admission	0.381	0.095	1.52	0.171
Emergency state	1.19	0.283	5.007	0.812
Appropriate empirical treatment	11.301	2.47	51.71	0.002
ST11	0.193	0.056	0.669	0.01

Figures

Figure 1

PFGE dendrogram generated with Bionumerics software showing the genetic relationship between 95 strains of carbapenem-resistant *Klebsiella pneumoniae*. The analysis of the bands generated was performed by using the Dice similarity coefficient and the unweighted pair group method with arithmetic averages.