Drug susceptibility and molecular epidemiology of Klebsiella pneumoniae bloodstream infection in ICU patients in Shanghai, China

Shuzhen Xiao
Department of Laboratory Medicine, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai

Tianchi Chen
Department of Laboratory Medicine, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai

Hairu Wang
Departments of Clinical Laboratory, Shanxi Provincial People’s Hospital, Affiliated of Shanxi Medical University, Taiyuan, Shanxi

Qing Chen
Department of Laboratory Medicine, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai

Feifei Gu
Department of Laboratory Medicine, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai

Zhitao Yang
Emergency Department, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai

Lizhong Han (✉ hanlizhong1107@163.com)
Department of Laboratory Medicine, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai

Erzhen Chen
Emergency Department, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai

Research Article

Keywords: Klebsiella pneumoniae, Bloodstream infections (BSIs)

DOI: https://doi.org/10.21203/rs.3.rs-122480/v1

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Abstract

Background Bloodstream infections (BSIs) are always associated with increased cost, prolonged hospitalization and higher mortality, especially for patients in intensive care units (ICUs). *Klebsiella pneumoniae* is recognized as the major cause of bacteremia around the world and resistant to most clinically significant antibiotics. This retrospective study focused on drug susceptibility and molecular epidemiology of *K. pneumoniae* isolated from ICU patients with BSI in Shanghai, China.

Methods Consecutive *K. pneumoniae* isolates were collected from ICU patients with bacteremia in Shanghai from January 2016 to December 2019. Antibiotic susceptibility testing and primary screening test for extended-spectrum β-lactamase (ESBL) and carbapenemase production were conducted by broth microdilution method. Polymerase chain reaction (PCR) was performed to detect antimicrobial resistance genes and to confirm carbapenemase production. We also conducted multilocus sequence typing (MLST), of which the result was analyzed by GoeBURST.

Results A total of 78 *K. pneumoniae* isolates were enrolled. *K. pneumoniae* isolated from ICU bloodstream infections (ICU-BSIs) were highly resistant to almost all clinically common antibiotics, except for colistin (11.5%) and tigecycline (23.0%). ESBL-producing and carbapenemase-producing *K. pneumoniae* accounted for 74.4% and 71.7%, respectively. The most frequently found genotype in ESBL producers was *bla*<sub>CTX-M-14</sub> (44/58, 75.9%), followed by *bla*<sub>CTX-M-15</sub> (15/58, 25.9%) and *bla*<sub>CTX-M-55</sub> (8/58, 13.8%). KPC is the only enzyme generated by carbapenemase producers and all KPC enzymes were encoded by *bla*KPC-2. The most principal ST was ST11 (50/78, 64.1%), followed by ST15 (7/78, 9.0%) and ST23 (3/78, 3.8%). Two newfound sequence types were identified in our study.

Conclusions This study is the first to demonstrate the antibiotic resistance phenotype and molecular epidemiology of *K. pneumoniae* isolated from ICU patients with bloodstream infections in Shanghai. It is noteworthy that ICU-BSI *K. pneumoniae* is characteristic of high resistance rate. According to the consequence of resistance gene detection and MLST analysis, the prevalence of KPC-2 enzyme may result from nosocomial clonal dissemination of ST11 *K. pneumoniae*.

1 Introduction

Bloodstream infections (BSIs) are recognized as a type of healthcare-associated infections (HAIs) of great clinical significance, attributing to the increased medical costs, prolonged hospital stays and higher mortality [1–3]. Patients in intensive care units (ICUs) have greater probability to suffer from BSIs, due to the high disease severity, immunosuppression status, invasive procedures, surgery and protracted hospitalization [4–6]. BSIs occur in approximately 5–15% of patients within the first month of ICU hospitalization [7]. Meanwhile, ICU-BSI is independently associated with a 40% increase in the risk of 30-day death [8].

*Klebsiella pneumoniae*, an environmental or opportunistic pathogen, is frequently associated with HAIs and is recognized as the major source for BSIs caused by gram-negative bacteria all over the world [6, 9, 10]. According to European Centre for Disease Prevention and Control, *K. pneumoniae* ranked third in the most common pathogens isolated in ICU-BSI episodes [11]. The SENTRY antimicrobial surveillance program which collected BSI organisms from more than 200 medical centers in 45 nations, demonstrated that the prevalence of *K. pneumoniae* increased consistently in all regions, especially in Europe (from 5.8–10.1%) and Asia-Pacific regions (from 7.6–13.5%) [12]. Consistent with these results, *K. pneumoniae* is the second most common bacteremia pathogen in China, based on a retrospective survey involving 10 cities [13]. Unfortunately, alongside with the high frequency, *K. pneumoniae* can also develop antibiotic resistance, with resistance to β-lactams being most clinically significant [9]. In China, *K.
pneumoniae was the most predominant pathogen in carbapenem-non-susceptible Enterobacteriaceae and the resistance rate of *K. pneumoniae* to carbapenem enhanced remarkably (from 3.0–25% in imipenem and from 2.9–26.3% in meropenem) [13, 14]. The carbapenem-non-susceptible *K. pneumoniae*, likely to be associated with production of carbapenemase, tends to have extensive drug resistant (XDR) phenotypes, leading to limited treatment options and poor outcomes [6, 15].

Appropriate and in-time antibiotic therapy which depends on epidemiology characteristics and drug susceptibility profiles, is critically important to the outcome of BSIs [16]. Although *K. pneumoniae* bloodstream infection in ICU patients is mortal, researches on this theme are still deficient. In this study, we collected the drug susceptibility results, explored the distribution of antibiotic resistance genes and analyzed the predominant sequence types (STs) of *K. pneumoniae* from ICU patients with BSIs in Shanghai. Such epidemiological data is useful to provide evidence for empirical therapy and develop strategies to prevent these serious infections.

**2 Materials And Methods**

**Setting and study design**

This retrospective and cross-sectional study of *K. pneumoniae* BSI in ICU patients, aiming to analyze drug susceptibility and molecular epidemiology of this pathogen, was performed in Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine. It is an 1800-bed comprehensive tertiary hospital located in Shanghai, a metropolitan region in China, with approximately 115,000 patient visits per year. Consecutive ICU patients with *K. pneumoniae* BSI were identified in the laboratory database of Department of Clinical Microbiology from January 2016 to December 2019. Only the first positive blood culture of each patient was recorded and enrolled in follow-up experiments.

**Microbiology identification and storage**

Identification of isolates from BSI patients in ICU was conducted on matrix-assisted laser desorption ionization-time of flight mass spectrometer (bioMérieux, Marcy-l’Étoile, France) and *K. pneumoniae* strains were stored in LB broth with 30% glycerol at -80°C for further experiments. LB broth and glycerol were bought from Sangon Biotech, Shanghai, China.

**Antibiotic Susceptibility Testing and Screening Test for Extended-spectrum β-lactamases (ESBLs) and Carbapenemases**

The result of drug susceptibility was acquired by the broth microdilution method using Sensititre™ GNX2F (Thermo Fisher Scientific, Waltham, MA, USA). Nineteen various antibiotics were involved in the trial, including amikacin, aztreonam, cefepime, cefotaxime, ceftazidime, ciprofloxacin, colistin, doxycycline, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, minocycline, piperacillin/tazobactam, ticarcillin-clavulanic acid, tigecycline, tobramycin and trimethoprim/sulfamethoxazole. *Pseudomonas aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as quality control in the antibiotics susceptibility assay. In this study, the result interpretation for tigecycline was based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. And other antibiotics were based on the Clinical and Laboratory Standard Institute (CLSI) 2019 guideline [17].

In accordance to CLSI 2019, screening test for ESBL production can be accomplished with ceftazidime and cefotaxime, while imipenem, ertapenem or meropenem could be used to screen for carbapenemase production. In this
study, ceftazidime, cefotaxime, ceftazidime-clavulanate, cefotaxime-clavulanate were used in screening and confirmatory test for ESBLs by the broth microdilution method. And isolates resistant to imipenem, ertapenem or meropenem were recorded as positive in carbapenemase production screening tests.

**DNA Extraction, Detection of Resistance Genes and Confirmation Test for Carbapenemases**

To obtain sample DNA from *K. pneumoniae*, bacteria were resuspended in distilled water and boiled at 100 °C for 15 min in order to lyse the cells and release DNA into aqueous phase. Sample DNA was then segregate from cell fragments through centrifugation at 3,000 g for 15 min and *K. pneumoniae* DNA was dissolved in the supernatant, which would be used as the origin of template DNA in PCR analysis. We detected the genotype of *K. pneumoniae* whose result was positive in the previous screening phase to clarify the causative genes that result in their ESBL and/or carbapenemase production. Seven genes associated with ESBLs were amplified, including *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX−M (−1, −2, −8, −9, −25 group)</sub>, *bla*<sub>OXA (−1, −2, −10 group)</sub>, *bla*<sub>VEB</sub>, *bla*<sub>GES</sub>, *bla*<sub>PER</sub>, together with several carbapenemase genes, such as *bla*<sub>VIM</sub>, *bla*<sub>IPM</sub>, *bla*<sub>KPC</sub>, *bla*<sub>GIM</sub>, *bla*<sub>IRM</sub>, *bla*<sub>SPM</sub>, *bla*<sub>SIM</sub>, *bla*<sub>OXA−48</sub> and *bla*<sub>NDM</sub>. Primers were used according to previous study [18]. Some drug-resistant isolates which were identified in previous study were used as positive control in PCR reaction [18–20]. After PCR amplification, the target products were separated through electrophoresis in 1% agarose gel. All positive products were sequenced and the genotypes were determined by comparing the sequencing results with the sequences in GeneBank (http://www.ncbi.nlm.nih.gov/BLAST).

**Multilocus Sequence Typing**

Multilocus sequence typing (MLST) of *K. pneumoniae* was based on seven conserved housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB*), which were amplified and sequenced in the study. After being aligned with MLST database (http://bigdweb.pasteur.fr/klebsiella/primers_used.html), each housekeeping gene sequence was associated with a unique allele and the combination of seven alleles determines the sequence type (ST) for each *K. pneumoniae* isolate. New alleles and STs discovered in our study were submitted to the curator of the database (klebsiellaMLST@pasteur.fr). GoEBURST was used for MLST analysis, demonstrating the allelic relationship and prevalence of various STs. In this study, isolates were classified into the same group if 6 of the 7 alleles were homologous.

**Statistical Analysis**

SPSS 25.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Values of categorical variables were presented as a percentage of the group where they derived. The chi-square test or Fisher's exact test was utilized to compare the classified variables, as appropriate. *P*<0.05 was considered to be statistically significant.

**3 Results**

**Characteristics of Total Patient Population**

From January 2016 to December 2019, a 48-month study period, a total of 78 consecutive *K. pneumoniae* isolates were consecutively collected from BSI patients in ICU. Among them, 18 isolates were obtained in 2016, 12 in 2017, 22 in 2018 and 26 in 2019. More males (54/78) than females (24/78) were enrolled in the study and the age of the 78 patients ranged from 17 to 91 years, with the median age of 60 years.

**Antimicrobial Susceptibility Tests**
The antimicrobial susceptibility of eligible *K. pneumoniae* to was displayed in Table 1. The highest resistance rate in *K. pneumoniae* isolated from BSI patients in ICU was 93.5% (resistant to ticarcillin-clavulanic acid and aztreonam), followed by 92.3% (resistant to ciprofloxacin and levofloxacin). In contrast, relatively low resistance appeared in colistin (11.5%) and tigecycline (23.0%). Among 78 *K. pneumoniae* isolates, 58 (74.4%) and 56 (71.7%) isolates were confirmed as ESBL-producers and carbapenemase-producers through gene detection, respectively. The ESBL-producing isolates exhibited statistically higher resistance to most antibiotics than non-ESBL-producing ones (*P* < 0.05), except for doxycycline, minocycline, trimethoprim-sulfamethoxazole, tigecycline and colistin, which was similar to carbapenemase producers. Furthermore, according to the consequences of susceptibility tests, *K. pneumoniae* isolates were classified into multidrug resistance (MDR, nonsusceptibility to ≥ 1 agent in ≥ 3 antimicrobial categories, 23/78, 29.5%), extensive drug resistance (XDR, susceptibility limited to ≤ 2 categories, 48/78, 61.5%) and pan-drug resistance (PDR, nonsusceptibility to all agents in all antimicrobial categories, 2/78, 2.6%).
Table 1
Rates of antibiotics resistance among *K. pneumoniae* BSIs in ICU patients

| Antimicrobial agents | Number of isolates (%) | *p*   | Number of isolates (%) | *p*   |
|----------------------|------------------------|-------|------------------------|-------|
|                      | Total (n = 78)         | ESBL (n = 58) | Non-ESBL (n = 20) | Carbapenemase (n = 56) | Non-carbapenemase (n = 22) |
| Ceftazidime          | 62(79.4)               | 58(100)  | 4(20.0)                 | 55(98.2)               | 7(31.8)                  | < 0.0001 |
| Cefotaxime           | 63(80.7)               | 58(100)  | 5(25.0)                 | 56(100)                 | 7(31.8)                  | < 0.0001 |
| Cefepime             | 62(79.4)               | 57(98.3) | 5(25.0)                 | 56(100)                 | 6(27.3)                  | < 0.0001 |
| Amikacin             | 67(85.8)               | 53(91.3) | 14(70.0)                | 52(92.8)                | 15(68.1)                 | 0.005    |
| Gentamicin           | 70(89.7)               | 56(96.6) | 14(70.0)                | 55(98.2)                | 15(68.1)                 | < 0.0001 |
| Tobramycin           | 70(89.7)               | 56(96.6) | 14(70.0)                | 55(98.2)                | 15(68.1)                 | < 0.0001 |
| Aztreonam            | 73(93.5)               | 57(98.3) | 16(80.0)                | 56(100)                 | 17(77.2)                 | < 0.0001 |
| Ciprofloxacin        | 72(92.3)               | 56(96.6) | 16(80.0)                | 56(100)                 | 16(72.7)                 | < 0.0001 |
| Levofloxacin         | 72(92.3)               | 56(96.6) | 16(80.0)                | 56(100)                 | 16(72.7)                 | < 0.0001 |
| Doxycycline          | 67(85.8)               | 50(86.2) | 17(85.0)                | 50(89.2)                | 17(77.2)                 | 0.170    |
| Ertapenem            | 55(70.5)               | 51(87.9) | 4(20.0)                 | 55(98.2)                | 0(0)                     | < 0.0001 |
| Imipenem             | 54(69.2)               | 51(87.9) | 3(15.0)                 | 54(96.4)                | 0(0)                     | < 0.0001 |
| Meropenem            | 55(70.5)               | 51(87.9) | 4(20.0)                 | 55(98.2)                | 0(0)                     | < 0.0001 |
| Minocycline          | 45(57.6)               | 34(58.6) | 11(55.0)                | 34(60.7)                | 11(50.0)                 | 0.389    |
| Piperacillin-tazobactam | 70(89.7)     | 56(96.6) | 14(70.0)                | 55(98.2)                | 15(68.1)                 | < 0.0001 |
| Ticarcillin-clavulanic acid | 73(93.5) | 57(98.3) | 16(80.0)                | 56(100)                 | 17(77.2)                 | < 0.0001 |
| Trimethoprim-sulfamethoxazole | 70(89.7)    | 54(93.1) | 16(80.0)                | 55(98.2)                | 15(68.1)                 | < 0.0001 |
| Tigecycline          | 18(23.0)               | 12(20.7) | 6(30.0)                 | 11(19.6)                | 7(31.8)                  | 0.251    |
| Colistin             | 9(11.5)                | 8(13.8)  | 1(5.0)                  | 9(16.0)                 | 0(0)                     | < 0.0001 |
Characterization of Resistance Genes

Among the 58 isolates with ESBL production, the predominant enzyme was CTX-M (57/58, 98.3%), followed by TEM (43/58, 74.1%). \textit{bla}_{\text{CTX-M-14}} (44/58, 75.9%) was the most frequently found genotype in ESBLs-producers, together with \textit{bla}_{\text{CTX-M-15}} (15/58, 25.9%) and \textit{bla}_{\text{CTX-M-55}} (8/58, 13.8%), which were relatively less common. All TEM enzymes were encoded by \textit{bla}_{\text{TEM-1}}. No \textit{bla}_{\text{CTX-M}} (−2, −8, −25 group), \textit{bla}_{\text{GES}}, \textit{bla}_{\text{VEB}}, \textit{bla}_{\text{OXA}} (−2, −10 group) or \textit{bla}_{\text{PER}} genes were found. Although \textit{bla}_{\text{SHV}} was detected in 40 strains and \textit{bla}_{\text{OXA}} (−1 group) in 8 strains, the sequencing results demonstrated that these genes were \textit{bla}_{\text{SHV-1}}, \textit{bla}_{\text{SHV-11}} and \textit{bla}_{\text{OXA-1}}, which belonged to \β-lactamase genes rather than ESBL genes. It was also worth noting that 44 of 58 ESBL producers harbored two or more ESBL genes.

KPC enzyme is the only carbapenemase produced by isolates resistant to carbapenem and all KPC enzymes were encoded by \textit{bla}_{\text{KPC-2}}, with no other carbapenemase genes detected in our study.

Multilocus Sequence Typing

Seventeen STs, including three new STs, were identified in 78 \textit{K. pneumoniae} isolates, among which the most principal STs was ST11 (50/78, 64.1%), followed by ST15 (7/78, 9.0%) and ST23 (3/78, 3.8%). All STs were clustered into one non-overlapping group (Fig. 1).

4 Discussion

As a critical nosocomial pathogen, \textit{K. pneumoniae} is one of the most common causative factors of BSIs and the worldwide dissemination of drug-resistant \textit{K. pneumoniae}, especially \β-lactam and carbapenem resistant \textit{K. pneumoniae}, has attracted global attention due to the limited treatment options and high mortality [18–20]. Alongside with its high drug-resistant rate, \textit{K. pneumoniae} is also increasingly associated with high virulence, which is called hypervirulent \textit{K. pneumoniae} and can cause severe infections, including liver abscesses and bacteremia [21]. Furthermore, \textit{K. pneumoniae} is the most frequent pathogen responsible for ICU bloodstream infections (ICU-BSIs), representing about 36.8% of all the ICU-BSIs in our hospital. Since ICU patients are predisposed to bacteremia, which can exert negative impact on the prognosis [22], our study focused on ICU-BSIs caused by \textit{K. pneumoniae} and intended to elucidate antibiotics susceptibility, resistance gene distribution and sequence types of these \textit{K. pneumoniae} so that clinicians can administer timely and appropriate antibiotics to improve the prognosis.

Consecutive ICU-BSI \textit{K. pneumoniae} isolates in Shanghai from January 2016 to December 2019 were enrolled in this study. Comparing with the previous study, which investigated the molecular epidemiology of BSI \textit{K. pneumoniae} from comprehensive source collected between 2012 and 2015, this recent research demonstrated that ICU-BSI \textit{K. pneumoniae} isolates harbored extremely higher resistance rate to nearly all commonly used antibiotics, such as ceftazidime, cefepime, cefotaxime, amikacin, gentamicin, tobramycin, aztreonam, ciprofloxacin, levofloxacin, piperacillin-tazobactam, imipenem, meropenem and trimethoprim-sulfamethoxazole [18]. It is also worth noting that the proportion of MDR (23/78, 29.5%) and XDR (48/78, 61.5%) in this study is higher than that in other literature [23]. While ICU-BSI \textit{K. pneumoniae} acquired great resistance to most antibiotics, it was relatively susceptible to tigecycline and colistin, which supported them as potential choices for empirical treatment of ICU-BSI caused by \textit{K. pneumoniae}. However, in critically ill patients, frequent administration of colistin and tigecycline were considered as risk factors for colistin and tigecycline-resistant \textit{K. pneumoniae} bloodstream infections, respectively [24]. Therefore, some new antibiotics have been developed, such as ceftazidime-avibactam, which can improve prognosis of bacteremia associated with carbapenem-resistant \textit{K. pneumoniae} deprived of metallo-\β-lactamase [25–27] and can decrease the usage of tigecycline and colistin to slow down evolution of antibiotic resistance.
In this study, the proportion of ESBL-producing *K. pneumoniae* isolated from ICU-BSI patients was 74.4%, much higher than our previous study (27.5%) [18], and also exceeded the proportion in Hongkong (12%), Thailand (27.4%) and American (15.5%) [28–30]. Despite the distinct ESBL-producing rate, the main type of ESBLs in *K. pneumoniae* was CTX-M enzyme in our study, consistent with the global trend [31]. Although \( \text{bla}_{\text{CTX-M-15}} \) was the dominant gene type in most regions worldwide, including India, Iran and Lebanon [19, 32, 33], \( \text{bla}_{\text{CTX-M-14}} \) was the most prevalent ESBL gene found in our study, followed by \( \text{bla}_{\text{CTX-M-55}} \) and \( \text{bla}_{\text{CTX-M-15}} \), which indicated that distribution of \( \text{bla}_{\text{CTX-M}} \) genes may be geographically different. Similarly, the rate of carbapenemase producer in ICU-BSI *K. pneumoniae* (71.8%) was higher than that in Taiwan (25%), Athens (60.7%) and Greece (62.3%) [33–35]. All carbapenemase-producing ICU-BSI *K. pneumoniae* isolates harbored \( \text{bla}_{\text{KPC-2}} \), similar to the study conducted in central China [36]. *K. pneumoniae* with KPC-2, comparing with NDM-1, was more resistant to amikacin, fosfomycin but more susceptible to trimethoprim/sulfamethoxazole and there were fewer appropriate treatment choices for KPC-2-producing *K. pneumoniae* [37]. According to the consequence of MLST, ST11 was the most predominant sequence type and 90% of them possessed \( \text{bla}_{\text{KPC-2}} \) gene, coinciding with other studies in China [6, 36]. The similarity of antibiotics resistance pattern and the prevalence of ST11 suggested that the nosocomial clonal dissemination of KPC-2-producing ST11 *K. pneumoniae* happened in ICU patients [38], indicating that current prevention strategies against *K. pneumoniae* in ICU should be adjusted and improved [39]. Moreover, ST11 was also dominant among hypervirulent carbapenemase-producing *K. pneumoniae*, which was frequently associated with severe infections [40, 41]. Thus, we are likely to pay attention to the virulence factors of ICU-BSI *K. pneumoniae* and find out whether the convergence of carbapenemase production and hypervirulence will exert negative effect on the outcomes of ICU-BSI patients in our following studies. It is noteworthy that two freshly new sequence types in our study harbored both ESBL and carbapenemase genes, suggesting the wide spread of the antibiotic resistance genes.

### 5 Conclusions

In conclusion, this retrospective study focused on drug susceptibility and molecular epidemiology of *K. pneumoniae* from ICU patients with BSI in Shanghai. Our data demonstrated that ICU-BSI *K. pneumoniae* isolates were highly resistant to clinically common antibiotics, except for tigecycline and colistin. Thus, it would be relatively appropriate to select tigecycline and colistin for empirical treatment. MLST and genetic analysis showed that nosocomial clonal dissemination of KPC-2-producing ST11 had already appeared in ICUs. Meanwhile, \( \text{bla}_{\text{CTX-M-14}} \) and \( \text{bla}_{\text{KPC-2}} \) were confirmed as the most prevalent ESBL and carbapenemase gene, respectively. These alert us that rational administration of antibiotics and regular surveillance of the molecular epidemiology of pathogens were urgent to impede the dissemination of such highly drug-resistant and mortal pathogen.

### Declarations

#### 7.1 Ethics approval and consent to participate

This study was approved by Ethics Committee of Ruijin Hospital affiliated to Shanghai Jiaotong University School of Medicine. The Review Board of Ruijin Hospital affiliated to Shanghai Jiaotong University School of Medicine waived request for informed consent because our study only put emphasis on bacteria and exerted no effect on patients. The Ethics Committee number is KY2019-147. All methods were performed in accordance with the relevant guidelines and regulations.

#### 7.2 Consent for publication

Not applicable
7.4 Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7.5 Funding

We acknowledge the support by the Shanghai Jiao Tong University School of Medicine Multicenter Clinical Research Program (DLY201803).

7.7 Acknowledgments

We are grateful to all the technicians of Clinical Microbiology in Ruijin Hospital for their support and assistance in bacteria collection and storage.

References

1. Yoon EJ, Choi MH, Park YS, Lee HS, Kim D, Lee H, et al. Impact of host-pathogen-treatment tripartite components on early mortality of patients with Escherichia coli bloodstream infection: Prospective observational study. *EBioMedicine* (2018) 35:76-86. Epub 2018/08/25. doi: 10.1016/j.ebiom.2018.08.029. PubMed PMID: 30139627; PubMed Central PMCID: PMCPMC6161478.

2. Zhu S, Kang Y, Wang W, Cai L, Sun X, Zong Z. The clinical impacts and risk factors for non-central line-associated bloodstream infection in 5046 intensive care unit patients: an observational study based on electronic medical records. *Crit Care* (2019) 23(1):52. Epub 2019/02/20. doi: 10.1186/s13054-019-2353-5. PubMed PMID: 30777109; PubMed Central PMCID: PMCPMC6379966.

3. Zimlichman E, Henderson D, Tamir O, Franz C, Song P, Yamin CK, et al. Health care-associated infections: a meta-analysis of costs and financial impact on the US health care system. *JAMA Intern Med* (2013) 173(22):2039-46. Epub 2013/09/04. doi: 10.1001/jamainternmed.2013.9763. PubMed PMID: 23999949.

4. Parajuli NP, Acharya SP, Mishra SK, Parajuli K, Rijal BP, Pokhrel BM. High burden of antimicrobial resistance among gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. *Antimicrob Resist Infect Control* (2017) 6:67. Epub 2017/06/24. doi: 10.1186/s13756-017-0222-z. PubMed PMID: 28638594; PubMed Central PMCID: PMCPMC5472869.

5. Timsit JF, Ruppe E, Barbier F, Tabah A, Bassetti M. Bloodstream infections in critically ill patients: an expert statement. *Intensive Care Med* (2020) 46(2):266-84. Epub 2020/02/13. doi: 10.1007/s00134-020-05950-6. PubMed PMID: 32047941; PubMed Central PMCID: PMCPMC7223992.

6. Zheng SH, Cao SJ, Xu H, Feng D, Wan LP, Wang GJ, et al. Risk factors, outcomes and genotypes of carbapenem-nonsusceptible Klebsiella pneumoniae bloodstream infection: a three-year retrospective study in a large tertiary hospital in Northern China. *Infect Dis (Lond)* (2018) 50(6):443-51. Epub 2018/01/06. doi: 10.1080/23744235.2017.1421772. PubMed PMID: 29303020.

7. Kallel H, Houcke S, Resiere D, Roy M, Mayence C, Mathien C, et al. Epidemiology and Prognosis of Intensive Care Unit-Acquired Bloodstream Infection. *Am J Trop Med Hyg* (2020). Epub 2020/04/22. doi: 10.4269/ajtmh.19-0877. PubMed PMID: 32314689.

8. Adrie C, Garrouste-Orgeas M, Ibn Essaied W, Schwebel C, Darmon M, Mourvillier B, et al. Attributable mortality of ICU-acquired bloodstream infections: Impact of the source, causative micro-organism, resistance profile and
antimicrobial therapy. J Infect (2017) 74(2):131-41. Epub 2016/11/14. doi: 10.1016/j.jinf.2016.11.001. PubMed PMID: 27838521.

9. Martin RM, Bachman MA. Colonization, Infection, and the Accessory Genome of Klebsiella pneumoniae. Front Cell Infect Microbiol (2018) 8:4. Epub 2018/02/07. doi: 10.3389/fcimb.2018.00004. PubMed PMID: 29404282; PubMed Central PMCID: PMCPMC5786545.

10. Wyres KL, Holt KE. Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Curr Opin Microbiol (2018) 45:131-9. Epub 2018/05/04. doi: 10.1016/j.mib.2018.04.004. PubMed PMID: 29723841.

11. Frerot M, Lefebvre A, Aho S, Callier P, Astruc K, Aho Glele LS. What is epidemiology? Changing definitions of epidemiology 1978-2017. PLoS One (2018) 13(12):e0208442. Epub 2018/12/12. doi: 10.1371/journal.pone.0208442. PubMed PMID: 30532230; PubMed Central PMCID: PMCPMC6287859.

12. Diekema DJ, Hsueh PR, Mendes RE, Pfaller MA, Rolston KV, Sader HS, et al. The Microbiology of Bloodstream Infection: 20-Year Trends from the SENTRY Antimicrobial Surveillance Program. Antimicrob Agents Chemother (2019) 63(7). Epub 2019/04/24. doi: 10.1128/AAC.00355-19. PubMed PMID: 31010862; PubMed Central PMCID: PMCPMC6591610.

13. Wang X, Zhao C, Li H, Chen H, Jin L, Wang Z, et al. [Microbiological profiles of pathogens causing nosocomial bacteremia in 2011, 2013 and 2016]. Sheng Wu Gong Cheng Xue Bao (2018) 34(8):1205-17. Epub 2018/08/29. doi: 10.13345/j.cjb.180192. PubMed PMID: 30152206.

14. Hu F, Guo Y, Yang Y, Zheng Y, Wu S, Jiang X, et al. Resistance reported from China antimicrobial surveillance network (CHINET) in 2018. Eur J Clin Microbiol Infect Dis (2019) 38(12):2275-81. Epub 2019/09/04. doi: 10.1007/s10096-019-03673-1. PubMed PMID: 31478103.

15. Piperaki ET, Syrogiannopoulos GA, Tzouvelekis LS, Daikos GL. Klebsiella pneumoniae: Virulence, Biofilm and Antimicrobial Resistance. Pediatr Infect Dis J (2017) 36(10):1002-5. Epub 2017/09/16. doi: 10.1097/INF.0000000000001675. PubMed PMID: 28914748.

16. Falcone M, Bassetti M, Tiseo G, Giordano C, Nencini E, Russo A, et al. Time to appropriate antibiotic therapy is a predictor of outcome in patients with bloodstream infection caused by KPC-producing Klebsiella pneumoniae. Crit Care (2020) 24(1):29. Epub 2020/02/01. doi: 10.1186/s13054-020-2742-9. PubMed PMID: 32000834; PubMed Central PMCID: PMCPMC6993311.

17. CLSI (2019). Performance Standards for Antimicrobial Susceptibility Testing, 29th Edn. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

18. Xiao SZ, Wang S, Wu WM, Zhao SY, Gu FF, Ni YX, et al. The Resistance Phenotype and Molecular Epidemiology of Klebsiella pneumoniae in Bloodstream Infections in Shanghai, China, 2012-2015. Front Microbiol (2017) 8:250. Epub 2017/03/11. doi: 10.3389/fmicb.2017.00250. PubMed PMID: 28280486; PubMed Central PMCID: PMCPMC5322179.

19. Chong Y, Shimoda S, Shimono N. Current epidemiology, genetic evolution and clinical impact of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Infect Genet Evol (2018) 61:185-8. Epub 2018/04/08. doi: 10.1016/j.meegid.2018.04.005. PubMed PMID: 29626676.

20. Wang B, Pan F, Wang C, Zhao W, Sun Y, Zhang T, et al. Molecular epidemiology of Carbapenem-resistant Klebsiella pneumoniae in a paediatric hospital in China. Int J Infect Dis (2020) 93:311-9. Epub 2020/02/19. doi: 10.1016/j.ijid.2020.02.009. PubMed PMID: 32068096.

21. Wang X, Xie Y, Li G, Liu J, Li X, Tian L, et al. Whole-Genome-Sequencing characterization of bloodstream infection-causing hypervirulent Klebsiella pneumoniae of capsular serotype K2 and ST374. Virulence (2018)
22. Bassetti M, Righi E, Carmelutti A. Bloodstream infections in the Intensive Care Unit. *Virulence* (2016) 7(3):267-79. Epub 2016/01/14. doi: 10.1080/21505594.2015.1134072. PubMed PMID: 26760527; PubMed Central PMCID: PMCPMC4871677.

23. Del Prete R, Ronga L, Addati G, Magrone R, Abbasciano A, Decimo M, et al. Trends in Klebsiella pneumoniae strains isolated from the bloodstream in a teaching hospital in southern Italy. *Infez Med* (2019) 27(1):17-25. Epub 2019/03/19. PubMed PMID: 30882374.

24. Papadimitriou-Olivgeris M, Bartzavali C, Spyropoulou A, Lambropoulou A, Sioulas N, Vamvakopoulou S, et al. Molecular epidemiology and risk factors for colistin- or tigecycline-resistant carbapenemase-producing Klebsiella pneumoniae bloodstream infection in critically ill patients during a 7-year period. *Diagn Microbiol Infect Dis* (2018) 92(3):235-40. Epub 2018/08/05. doi: 10.1016/j.diagmicrobio.2018.06.001. PubMed PMID: 30076041.

25. Shields RK, Nguyen MH, Chen L, Press EG, Potoski BA, Marini RV, et al. Ceftazidime-Avibactam Is Superior to Other Treatment Regimens against Carbapenem-Resistant Klebsiella pneumoniae Bacteremia. *Antimicrob Agents Chemother* (2017) 61(8). Epub 2017/06/01. doi: 10.1128/AAC.00883-17. PubMed PMID: 28559250; PubMed Central PMCID: PMCPMC5527595.

26. Zhang W, Guo Y, Li J, Zhang Y, Yang Y, Dong D, et al. In vitro and in vivo bactericidal activity of ceftazidime-avibactam against Carbapenemase-producing Klebsiella pneumoniae. *Antimicrob Resist Infect Control* (2018) 7:142. Epub 2018/11/28. doi: 10.1186/s13756-018-0435-9. PubMed PMID: 30479755; PubMed Central PMCID: PMCPMC6249859.

27. Mavroidi A, Katsiari M, Likousi S, Palla E, Roussou Z, Nikolaou C, et al. Changing Characteristics and In Vitro Susceptibility to Ceftazidime/Avibactam of Bloodstream Extensively Drug-Resistant Klebsiella pneumoniae from a Greek Intensive Care Unit. *Microb Drug Resist* (2020) 26(1):28-37. Epub 2019/08/07. doi: 10.1089/mdr.2019.0090. PubMed PMID: 31386596.

28. Sawatwong P, Sapchookul P, Whistler T, Gregory CJ, Sangwichian O, Makprasert S, et al. High Burden of Extended-Spectrum beta-Lactamase-Producing Escherichia coli and Klebsiella pneumoniae Bacteremia in Older Adults: A Seven-Year Study in Two Rural Thai Provinces. *Am J Trop Med Hyg* (2019) 100(4):943-51. Epub 2019/02/23. doi: 10.4269/ajtmh.18-0394. PubMed PMID: 30793684; PubMed Central PMCID: PMCPMC6447101.

29. Abodakpi H, Chang KT, Sanchez Diaz AM, Canton R, Lasco TM, Chan K, et al. Prevalence of extended-spectrum beta-lactamase and carbapenemase-producing bloodstream isolates of Klebsiella pneumoniae in a tertiary care hospital. *J Chemother* (2018) 30(2):115-9. Epub 2017/11/11. doi: 10.1080/1120009X.2017.1399233. PubMed PMID: 29125052.

30. Man MY, Shum HP, Chan YH, Chan KC, Yan WW, Lee RA, et al. Clinical predictors and outcomes of Klebsiella pneumoniae bacteraemia in a regional hospital in Hong Kong. *J Hosp Infect* (2017) 97(1):35-41. Epub 2017/06/13. doi: 10.1016/j.jhin.2017.06.007. PubMed PMID: 28602703.

31. Calbo E, Garau J. The changing epidemiology of hospital outbreaks due to ESBL-producing Klebsiella pneumoniae: the CTX-M-15 type consolidation. *Future Microbiol* (2015) 10(6):1063-75. Epub 2015/06/11. doi: 10.2217/fmb.15.22. PubMed PMID: 26059626.

32. Obeid A, Maliha P, Abdallah S, Akl E, Deeb M, El Moussawi H, et al. ESBL-producing Escherichia coli and Klebsiella pneumoniae in two major Lebanese hospitals: molecular epidemiology and correlation with consumption. *J Infect Dev Ctries* (2018) 12(2.1):16S. Epub 2018/02/22. doi: 10.3855/jidc.10038. PubMed PMID: 31804991.
33. Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R. Carbapenem resistant Klebsiella pneumoniae isolated from bloodstream infection: Indian experience. *Pathog Glob Health* (2017) 111(5):240-6. Epub 2017/07/04. doi: 10.1080/20477724.2017.1340128. PubMed PMID: 28670975; PubMed Central PMCID: PMC560201.

34. Lin YT, Su CF, Chuang C, Lin JC, Lu PL, Huang CT, et al. Appropriate Treatment for Bloodstream Infections Due to Carbapenem-Resistant Klebsiella pneumoniae and Escherichia coli: A Nationwide Multicenter Study in Taiwan. *Open Forum Infect Dis* (2019) 6(2):ofy336. Epub 2019/02/12. doi: 10.1093/ofid/ofy336. PubMed PMID: 30740468; PubMed Central PMCID: PMCPMC6362312.

35. Daikos GL, Tsaousi S, Tzouvelekis 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. *Antimicrob Agents Chemother* (2014) 58(4):2322-8. Epub 2014/02/12. doi: 10.1128/AAC.02166-13. PubMed PMID: 24514083; PubMed Central PMCID: PMCPMC4023796.

36. Li Y, Shen H, Zhu C, Yu Y. Carbapenem-Resistant Klebsiella pneumoniae Infections among ICU Admission Patients in Central China: Prevalence and Prediction Model. *Biomed Res Int* (2019) 2019:9767313. Epub 2019/04/30. doi: 10.1155/2019/9767313. PubMed PMID: 31032370; PubMed Central PMCID: PMCPMC6457282.

37. Lin L, Xiao X, Wang X, Xia M, Liu S. In Vitro Antimicrobial Susceptibility Differences Between Carbapenem-Resistant KPC-2-Producing and NDM-1-Producing Klebsiella pneumoniae in a Teaching Hospital in Northeast China. *Microb Drug Resist* (2020) 26(2):94-9. Epub 2019/08/23. doi: 10.1089/mdr.2018.0398. PubMed PMID: 31433255.

38. Gu B, Bi R, Cao X, Qian H, Hu R, Ma P. Clonal dissemination of KPC-2-producing Klebsiella pneumoniae ST11 and ST48 clone among multiple departments in a tertiary teaching hospital in Jiangsu Province, China. *Ann Transl Med* (2019) 7(23):716. Epub 2020/02/12. doi: 10.21037/atm.2019.12.01. PubMed PMID: 32042732; PubMed Central PMCID: PMCPMC6990001.

39. Liu J, Yu J, Chen F, Yu J, Simner P, Tamma P, et al. 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(2):293-9. Epub 2017/12/29. doi: 10.1007/s10096-017-3131-4. PubMed PMID: 29282569; PubMed Central PMCID: PMCPMC5780533.

40. Karlsson M, Stanton RA, Ansari U, McAllister G, Chan MY, Sula E, et al. Identification of a Carbapenemase-Producing Hypervirulent Klebsiella pneumoniae Isolate in the United States. *Antimicrob Agents Chemother* (2019) 63(7). Epub 2019/05/08. doi: 10.1128/AAC.00519-19. PubMed PMID: 31061159; PubMed Central PMCID: PMCPMC6591612.

41. Pan H, Lou Y, Zeng L, Wang L, Zhang J, Yu W, et al. Infections Caused by Carbapenemase-Producing Klebsiella pneumoniae: Microbiological Characteristics and Risk Factors. *Microb Drug Resist* (2019) 25(2):287-96. Epub 2019/02/28. doi: 10.1089/mdr.2018.0339. PubMed PMID: 30810470; PubMed Central PMCID: PMCPMC6441289.