Resistance phenotype and molecular epidemiology of Carbapenem-resistant Klebsiella pneumoniae isolates in Shanghai

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Abstract

Background: The emergence and wide global spread of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates are of great concern, and the aim of this study was to investigate drug resistance, molecular epidemiology, and genetic relationship of CRKP isolates from patients in Shanghai, China.

Methods: A retrospective study was conducted from April 2018 to July 2019, and a total of 133 CRKP isolates were collected. Antimicrobial susceptibility was determined by VITEK-2 automated microbiology analyzer platform (bioMérieux, France) and the broth microdilution method. Polymerase chain reaction (PCR) assays were used to investigate the presence of drug resistance genes. A modified carbapenem inactivation method (mCIM) was performed to detect carbapenemases. Multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) were conducted for genetic relatedness of 50 CRKP isolates selected.

Results: Among 670 isolates of *K. pneumoniae*, 133 (19.85%) strains were identified as carbapenem-resistant *K. pneumoniae* (CRKP), of which, 76.69% (102/133) strains were isolated from ICUs. All the 133 CRKP isolates were found to be carbapenemase-producers and harbor bla*KPC-2* gene. No other carbapenemase genes of *blaNDM*, *blaOXA−48*, *blaVIM*, and *blaIMP* were detected. Furthermore, β-lactamase genes of *blaSHV*, *blaCTX*, and *blaTEM* were the most common resistance-associated genes among these KPC-2 producing isolates. All the 133 CRKP strains displayed more than 95% of resistance to cephalosporins and carbapenems, except for gentamicin, Trimethoprim-sulfamethoxazole, amikacin, tigecycline and colistin. The most common sequence type was ST11, accounting for 90.0% of the 50 CRKP selected, followed by ST15 (10%). PFGE analysis clustered the 50 *KPC-2*-producing isolates into seven (A-G) distinct clonal clusters at 85% cut off. Of which, cluster A and G were the two major clusters, accounting for the majority of the strains collected in emergency ICU and neurosurgical ICU. And all the strains of cluster D and E were collected in cardiothoracic surgery ICU, expect for one strain collected in one outpatient.

Conclusion: The *KPC-2*-producing *K.pneumoniae* belonged to ST11 was widely disseminated in ICUs, and active and effective surveillance of infection control strategies was initiated to limit the spread of CRKP strains.

Introduction

Klebsiella pneumoniae is a common nosocomial infection pathogen in hospitals, especially the infection caused by carbapenem-resistant *K. pneumoniae* (CRKP) clinical isolates has spread widely throughout the world[1−4]. According to the results of 12-year bacterial resistance monitoring from China Antimicrobial Surveillance Network (CHINET), the significant rising resistance trend of *K. pneumoniae* to impenem or meropenem, from 3.0% and 2.9% in 2005 to 25.0% and 26.3% in 2018[5]. Since the CRKP strain is usually resistant to the most antimicrobials commonly used in clinical practice, few therapeutic

options are remaining. Therefore, treatment of CRKP infections are extremely challenging due to its high levels of morbidity and mortality[6], especially for patients in intensive care unit (ICU).

The main cause of carbapenem resistance is the production of carbapenemase such as *K. pneumoniae* carbapenemases (KPCs), New Delhi metallo-β-lactamase (NDM) and oxacillinase type 48 (OXA-48). In China, KPC-producing *K. pneumoniae* strains were the most predominant in adults, while the NDM-producing strains were mainly isolated in children[7]. The both carbapenemase genes are predominantly plasmid encoded, which facilitated the highly transmission across multiple enterobacterial species. Therefore, understanding the resistance phenotype and molecular epidemiology of CRKP isolates is essential to control the wide spread of these strains and promoting the selection and efficacy of empirical therapy.

Thus, a retrospective study in our hospital from April 2018 to July 2019 focusing on the molecular epidemiology of CRKP strains was conducted to provide reference for clinical anti-infection treatment and the control of nosocomial infection.

Methods

Bacterial isolates

From April 2018 to July 2019, a total of 133 carbapenem-resistant *K. pneumoniae* isolates were consecutively collected in Shuguang Hospital, Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China. These 133 strains represented 19.85% of all isolates of 670 non-duplicated *K. pneumoniae* cultured form patients during the study period. All isolates were identified by the VITEK-2 system.

Antimicrobial susceptibility testing and phenotypic analysis
Antimicrobial susceptibility testing was performed by VITEK-2 system and broth microdilution method, and the results were interpreted according to 2018 Clinical and Laboratory Standards Institute(CLSI) guidelines[8] except for colistin and tigecycline, which according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[9] interpretive criteria(http://www.eucast.org/). Escherichia coli ATCC 25922 was used as the quality control for antimicrobial susceptibility testing. The definition of a CRKP strain is resistant to imipenem or meropenem. A modified carbapenem inactivation method (mCIM) was performed to detect carbapenemases, as described previously[10].
Detection of antimicrobial resistance genes
Polymerase chain reaction (PCR) assays were performed as described previously to investigate the presence of drug resistance genes, including carbapenemase-encoding genes (*blaKPC, blaNDM, blaOXA − 48, blaVIM, and blaIMP*), AmpC genes (*DHA, FOX, AAC, EBC*, and *CIT*) and extended-spectrum β-lactamase (ESBL)-encoding genes (*blaCTX, blaSHV, and blaTEM*)[11−14].
Pulsed-field gel electrophoresis (PFGE)

Genetic relatedness among CRKP strains was performed using PFGE method according to the protocol outlined in the PulseNet for Enterobacteriaceae. In brief, the whole genomic DNA was prepared by agarose embedding method, followed by XbaI digestion with the Bio-Rad CHEF Mapper electrophoresis system. Salmonella serotype Braenderup strain H9812 was used as a control strain and molecular marker. The PFGE banding pattern was clustered by BioNumerics software using the Dice Similarity coefficient.

Multilocus sequence typing (MLST)

MLST was performed by amplifying the seven conserved *K. pneumoniae* housekeeping genes, including *gapA, infB, mdh, pgi, phoE, rpoB*, and tonB. Alleles and sequence types (STs) were assigned according to the protocol available on the Institute Pasteur MLST database website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).

Results

Clinical characteristics

Among 670 isolates of *K. pneumoniae*, 133 (19.85%) strains were identified as carbapenem-resistant *K. pneumoniae* (CRKP). Most CRKP isolates were detected in the intensive care unit (76.69%, 102/133), including emergency ICU, neurosurgical ICU, cardiothoracic surgery ICU, neurological ICU and respiratory ICU, followed by neurosurgery wards (8.27%, 11/133) and gastroenterology wards(6.77%, 9/133). And the majority of CRKP strains were isolated from sputum (60.90%, 81/133), followed by bile (9.02%, 12/133), urine (9.02%, 12/133), blood (6.02%, 8/133), and aseptic body fluids samples (5.26%, 7/133) such as cerebrospinal fluid, BALF and drainage.

Antimicrobial Susceptibility

The antimicrobial resistance rates of the 133 CRKP isolates were shown in Table 1. They displayed more than 95% of resistance to cephalosporins and carbapenems, apart from gentamicin, Trimethoprim-sulfamethoxazole, amikacin, tigecycline and Colistin.

Table 1
Resistance to antimicrobial agents of 133 CRKP isolates

Antimicrobial Agent	R(%)	I(%)	S(%)
Cefazolin	100.0	0.0	0.0
Cefuroxime	100.0	0.0	0.0
Ceftazidime	100.0	0.0	0.0
Cefotaxime	100.0	0.0	0.0
Cefatriaxone	100.0	0.0	0.0
Cefepime	100.0	0.0	0.0
Piperacillin-tazobactam	100.0	0.0	0.0
Ampicillin-sulbactam	100.0	0.0	0.0
Imipenem	100.0	0.0	0.0
Meropenem	99.2	0.8	0.0
Cefoperazone-sulbactam	99.2	0.0	0.8
Levofloxacin	98.5	0.8	0.8
Gentamicin	75.2	2.3	22.6
Trimethoprim-sulfamethoxazole	72.9	0.0	27.1
Ampicillin	30.1	0.8	69.2
Tegafycline	3.0	0.0	97.0
Colistin	2.3	0.0	97.7
R Resistant, I Intermediary, S Susceptible			

Molecular Characteristics of CRKP Isolates

All 133 CRKP were found to be carbapenemase-producers determined by modified carbapenem inactivation method (mCIM). And the carbapenemase gene of *blaKPC-2* was detected in all these strains, which was in accordance with the phenotypic results. No other carbapenemase genes of *blaNDM*, *blaOXA – 48*, *blaVIM*, and *blaIMP* were detected. In addition to *blaKPC-2* gene, β-lactamase genes of *blaSHV*, *blaCTX*, and *blaTEM* were identified in 88.7% (118/133), 55.6% (74/133) and 77.4% (103/133) isolates, respectively. While, only 4 strains were positive for gene *DHA*, encoding plasmid-mediated AmpCs.

Of the 133 *blaKPC-2*-producing *Klebsiella pneumoniae* isolates, PFGE and MLST analysis were further performed on a subset of 50 isolates. Isolates were selected according to the respective proportions,

specimen types and isolated intervals of these blaKPC-2-positive *K. pneumoniae* strains per ward, to provide a representative image of the different wards.

MLST analysis showed that the most common sequence type was ST11, accounting for 90.0% of the 50 CRKP selected, followed by ST15 (10.0%). PFGE analysis revealed seven distinct clusters and related sub-clusters of the 50 blaKPC-2-positive isolates with 85% and 95% cutoff genetic similarity, relatively, as shown in Fig. 1. All the tested isolates of PFGE clusters A-F were belonged to MLST type ST11 and only five isolates of PFGE cluster G were belonged to MLST type ST15. Furthermore, cluster A and G appeared dominantly represented, comprising 16 (32%) isolates and 22(44%) isolates, respectively. Of which, cluster A accounted for the majority of the strains collected in emergency ICU and cluster G accounted for isolates mostly in neurosurgical ICU and emergency ICU. And all the strains of cluster D and E were collected in cardiothoracic surgery ICU, expect for one strain collected in one outpatient.

Discussion

Intensive Care Unit (ICU) is the main department of nosocomial infections and has been considered as a factory for manufacturing, disseminating, and enhancing antimicrobial resistance[15]. The increasing incidence of antimicrobial resistance of isolates from ICU is becoming more and more serious due to the complicated conditions such as extremely vulnerable critically ill patients with many basic diseases, use of invasive operations, relatively long hospitalization time and excessive use of generally broad-spectrum antimicrobials, which bring difficulties to clinical anti-infective treatment[16, 17]. Meanwhile, the infections caused by CRKP are significantly increasing over time[18], which pose a challenge to clinical treatment and negative impact on patient outcome due to the limited availability of clinically antimicrobials.

In our study, among 133 CRKP strains, 102 were isolated in the intensive care unit, accounting for 76.69%, showing the high prevalence of patients, which was consistence with previous studies[19, 20]. Additional, the isolation rate of CRKP in sputum was the highest (60.90%), which might be due to the heavy use of ventilator reported as the major risk factor of CRKP infections in ICUs.

All the 133 CRKP isolates were found to be carbapenemase-producers and harbor bla*KPC-2* gene, which accounted for the most frequent mechanism for resistance to carbapenems in *K. pneumoniae*. In China, KPC-producing-*K.pneumoniae* was the most prevailing and reported as the predominant outbreak strain[21, 22]. Fortunately, none multi-carbapenemase-producing K.pneumoniae was observed in this study. However, further analysis revealed the high prevalence of the combination of three β-lactamases genes of *blaSHV, blaCTX*, and *blaTEM* among these KPC-2 producing strains. The co-existence of resistant determinants with the combination of carbapenemase and two or more β-lactamases leaded to the multi-drug resistance.

As a result, all the isolates displayed a multi-resistant phenotype, with all resistant to cephalosporins and carbapenems, such as cefazolin, cefuroxime, cefatriaxone, ceftazidime, cefotaxime, cefepime, piperacillin-tazobactam, ampicillin-sulbactam, and imipenem. Although only 3.0% and 2.3% isolates

showed resistance to tegafycline and Colistin, respectively, the two antimicrobials had the disadvantages of low plasma concentration and significant toxicity. Thus, treatment of infections caused by these multi-resistant isolates are extremely challenging due to its limited antimicrobials and high levels of morbidity and mortality.

According to MLST typing, ST11 was the most dominant sequence type of the 50 CRKP isolates, accounting for 90.0%, which was consistent with previous studies in China[15, 19]. In addition, ST11 clone is the single locus variant of ST258, and that they belong to the same clonal members of CG258[23], which has contributed largely to the global dissemination of KPC carbapenemase over the past 20 years[24, 25]. The difference is that the ST258 clone is prevailing in North America and Europe, while the ST11clone is frequently isolated in Asia[25]. Moreover, ST11 clone showed multidrug-resistant phenotype with high carriage rate of virulence genes. These virulence factors are conducive to binding, biofilm formation, colonization, and anti-phagocytosis, enhancing environmental survival and, which can successfully demonstrate their persistence and quick expansion across the world[26, 27] .

In addition to ST11, five ST15 *KPC*-2-producing *K. pneumoniae* were isolated in patients in neurosurgical ICU, gastroenterology ward, emergency ICU and outpatient, relatively. It was worth noting that *K. pneumoniae* isolates of ST15 was widely disseminated in Europe and repeatedly reported to spread carbapenemase genes such as NDM-1 in North African[28] and in Greece[29] and OXA-48 in Spain[30]. However, ST15 is gradually detected in China[31, 32]. Moreover, in our study one ST15 strain was isolated from one outpatient who had not been hospitalized or received health care services in the previous 3 months, suggesting that ST15 is a potential risk clone to be a community onset which deserves routine surveillance to prevent outbreak.

MLST is appropriate for genotyping to further provide data not only for local epidemiological investigations but also for global and evolutionary studies. However, since MLST only involve seven housekeeping genes, it is less discriminating than PFGE to analyze outbreaks in different wards. In our study, all the ST11 isolates were divided into A-G seven different PFGE clusters and related sub-clusters. Of which, sub-cluster A1 and G1 appeared the two most predominant clones with > 95% similarity of PFGE patterns. And the sub-cluster A1 consisted of all the isolates collected in emergency ICU, expect for two isolates in neurosurgical, and sub-cluster G1 in both neurosurgical ICU and emergency ICU, suggesting that the two prevalent clones were transmitted in the two ICUs. In addition, there were two isolates assigned to cluster B and one isolate belonged to cluster F were also detected in emergency ICU, which suggested us that emergency ICU was one of the most high-risk wards and might be the focal point of active monitoring for infection control.

Finally, the six isolates belonged to cluster D and E were observed just in cardiothoracic surgery ICU, except one in an outpatient, which might had different genetic backgrounds from the isolates in neurosurgical ICU and emergency ICU. It was worth noting that the CRKP isolates assigned to cluster A, B, C, E and G all had the ability to cause blood infection, which deserved our attention.

In conclusion, our study revealed that ICUs were the main wards for rapid and wide spread of CRKP and clonal dissemination and outbreak of KPC-2-producing CRKP belonged to ST11 was observed in ICUs, especially in emergency ICU in our hospital. Hence,routine surveillance and effective infection control strategies are urgently warranted on these high-risk international strains to prevent outbreak and nosocomial-acquired infection.

Abbreviations

CRKP
carbapenem-resistant *Klebsiella pneumoniae*
PCR
Polymerase chain reaction
mCIM
modified carbapenem inactivation method
MLST
Multilocus sequence typing
PFGE
pulsed-field gel electrophoresis
ICU
Intensive Care Unit

Declarations

Ethics approval and consent to participate:

This study was approved by Shuguang hospital.

Consent for publication:

Not applicable.

Availability of data and material:

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

Competing interests:

We have no conflicts of interest to declare.

Funding:

Authors' contributions:

All authors read and approved the final version of the manuscript.

Acknowledgements:

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Figures

PFGE-XbaI   Kb

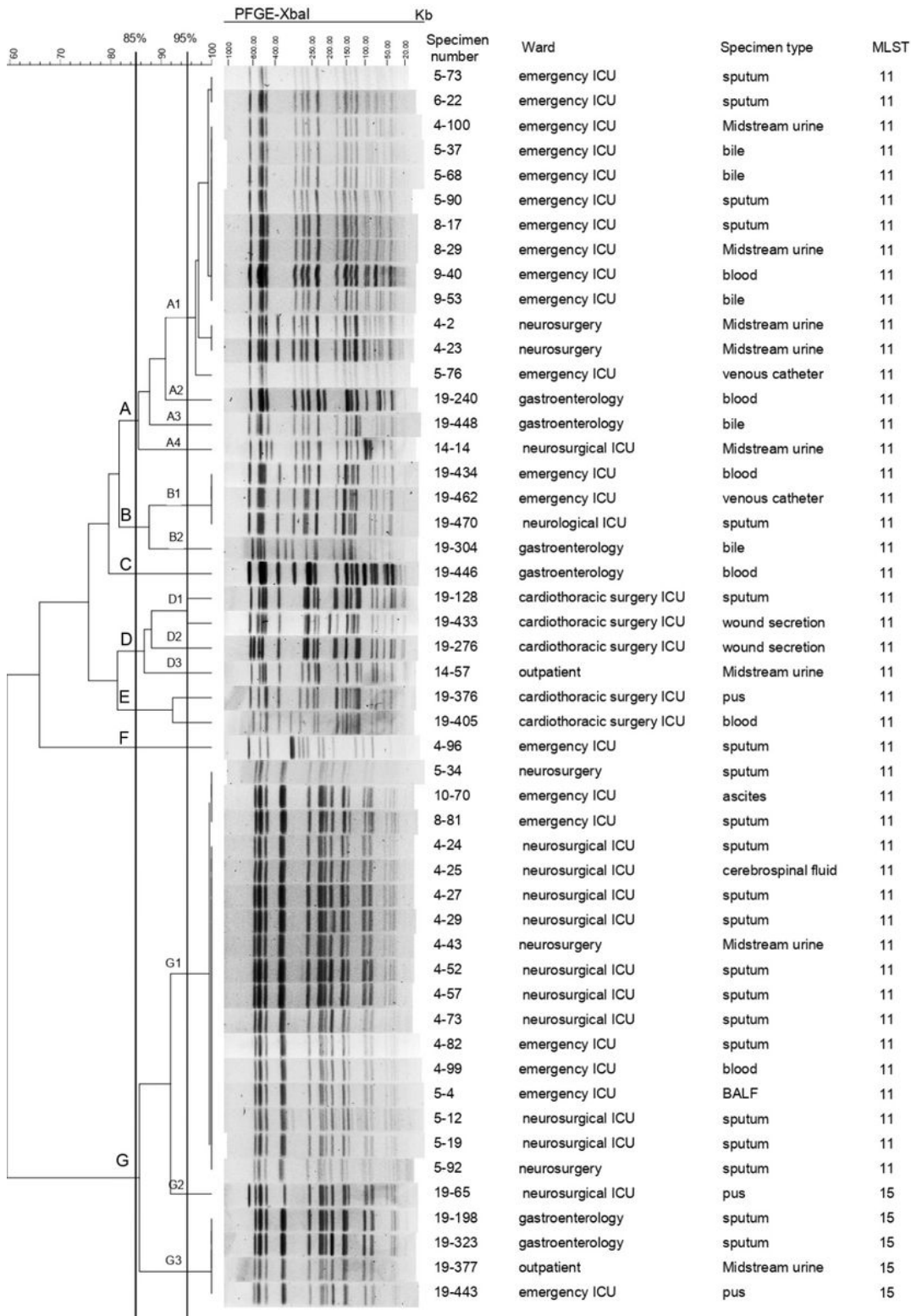| Specimen number	Ward	Specimen type	MLST
5-73	emergency ICU	sputum	11
6-22	emergency ICU	sputum	11
4-100	emergency ICU	Midstream urine	11
5-37	emergency ICU	bile	11
5-68	emergency ICU	bile	11
5-90	emergency ICU	sputum	11
8-17	emergency ICU	sputum	11
8-29	emergency ICU	Midstream urine	11
9-40	emergency ICU	blood	11
9-53	emergency ICU	bile	11
4-2	neurosurgery	Midstream urine	11
4-23	neurosurgery	Midstream urine	11
5-76	emergency ICU	venous catheter	11
19-240	gastroenterology	blood	11
19-448	gastroenterology	bile	11
14-14	neurosurgical ICU	Midstream urine	11
19-434	emergency ICU	blood	11
19-462	emergency ICU	venous catheter	11
19-470	neurological ICU	sputum	11
19-304	gastroenterology	bile	11
19-446	gastroenterology	blood	11
19-128	cardiothoracic surgery ICU	sputum	11
19-433	cardiothoracic surgery ICU	wound secretion	11
19-276	cardiothoracic surgery ICU	wound secretion	11
14-57	outpatient	Midstream urine	11
19-376	cardiothoracic surgery ICU	pus	11
19-405	cardiothoracic surgery ICU	blood	11
4-96	emergency ICU	sputum	11
5-34	neurosurgery	sputum	11
10-70	emergency ICU	ascites	11
8-81	emergency ICU	sputum	11
4-24	neurosurgical ICU	sputum	11
4-25	neurosurgical ICU	cerebrospinal fluid	11
4-27	neurosurgical ICU	sputum	11
4-29	neurosurgical ICU	sputum	11
4-43	neurosurgery	Midstream urine	11
4-52	neurosurgical ICU	sputum	11
4-57	neurosurgical ICU	sputum	11
4-73	neurosurgical ICU	sputum	11
4-82	emergency ICU	sputum	11
4-99	emergency ICU	blood	11
5-4	emergency ICU	BALF	11
5-12	neurosurgical ICU	sputum	11
5-19	neurosurgical ICU	sputum	11
5-92	neurosurgery	sputum	11
19-65	neurosurgical ICU	pus	15
19-198	gastroenterology	sputum	15
19-323	gastroenterology	sputum	15
19-377	outpatient	Midstream urine	15
19-443	emergency ICU	pus	15

Figure 1

Relationships of the 50 KPC-2-producing K. pneumoniae isolates based on Pulsed Field Gel Electrophoresis (PFGE) and MLST