A Polyclonal Spread Emerged: Characteristics of Carbapenem-Resistant *Klebsiella pneumoniae* Isolates from the Intensive Care Unit in a Chinese Tertiary Hospital

ZHENGZHENG WANG1,2, FANGYOU YU3,4, XIAOFEI SHEN5 and MEILAN LI*6

1 Department of Clinical Laboratory, Hwa Mei Hospital, University of Chinese Academy of Sciences, Ningbo, Zhejiang, China
2 Ningbo Institute of Life and Health, University of Chinese Academy of Sciences, Ningbo, Zhejiang, China
3 Department of Laboratory Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China
4 Shanghai Key Laboratory of Tuberculosis, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China
5 Department of Respiratory Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China
6 Emergency Intensive Care Unit, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China

Submitted 31 March 2020, revised 8 July 2020, accepted 22 July 2020

**Abstract**

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates often cause nosocomial infections with limited therapeutic options and spread rapidly worldwide. In this study, we revealed a polyclonal emergence of CRKP isolates from the intensive care unit in a Chinese tertiary hospital. We applied a series of methods including automated screening, antimicrobial susceptibility testing, the modified carbapenem inactivation method (mCIM), PCR amplification, DNA sequencing, and multilocus sequence typing (MLST) to characterize 30 non-duplicated CRKP isolates along with the collection of the related medical records. The results showed the polyclonal spread of CRKP isolates belonged to ST722, ST1446, ST111, ST896, ST290, and ST11. Among them, ST722 and ST1446 were two novel types of *K. pneumoniae*, and ST896 isolate harboring *bla*KPC-2 was also found for the first time. Since the polyclonal spread of CRKP in the same ward is rare, the silent clonal evolution with the switching genotypes prompts us to stay alert for outbreaks caused by novel subclones.

**Keywords:** polyclonal spread, carbapenem-resistant *Klebsiella pneumoniae*, sequence type, intensive care unit, alert

**Introduction**

*Klebsiella pneumoniae* is regarded as an opportunistic Gram-negative pathogen that can cause several infections such as pneumonia, urinary tract infections, and bloodstream infections (Magill et al. 2014). Due to the overuse of carbapenems for treating severe infections caused by extended-spectrum β-lactamases (ESBLs)-producing bacteria, carbapenem-resistant *K. pneumoniae* (CRKP) has rapidly increased globally in the past decade (Logan and Weinstein 2017). The expression of plasmid-mediated carbapenemases has been the primary mechanism of carbapenem resistance, and *K. pneumoniae* carbapenemase (KPC) is the most frequent type found in this species (Martin and Bachman 2018). Among these isolates, ST11 was the predominant clone responsible for disseminating the resistance gene *bla*KPC-2 in China (Qi et al. 2011), whereas ST258 accounted for the large majority of KPC-producing *K. pneumoniae* in the world (Kitchel et al. 2009; Hammerum et al. 2010). Moreover, additional types of carbapenemases have also emerged in *K. pneumoniae* like NDM-1 and OXA-48 categorized as class B metallo-β-lactamase (MBL) and class D enzymes, respectively, which confer specific levels of resistance to carbapenems. Ever since NDM-1 was discovered in *K. pneumoniae* isolate collected from a Swedish patient who had been hospitalized in India in 2008 (Yong et al. 2009), twenty-four NDM variants have been identified. It poses a significant threat...
to public health and a severe challenge for clinical treatments (Wu et al. 2019).

ICU hospitalization itself has been considered as an independent risk factor for CRKP acquisition (Schwaber et al. 2008; Hussein et al. 2009; Debby et al. 2012). The estimated detection rate of CRKP in patients admitted to intensive care units increased by 75% in a 20-year surveillance study in China (Tian et al. 2019). The gastrointestinal carriage rate of CRKP among ICU patients could reach 39.0–74.5%. It can be recognized as a reservoir of CRKP for progression from colonization to infection and the potential route of transmission of carbapenem resistance genes (Bratu et al. 2005; Snitkin et al. 2012; Papadimitriou-Olivgeris et al. 2013). Additionally, ICU is often deemed the epicenter of nosocomial infections caused by multidrug-resistant organisms (MDRO) due to the burdens of the vulnerable populations of critically immunocompromised patients and multiple invasive procedures. Thus, the outcome of patients with CRKP infections is inferior, leading to higher mortality in the setting of ICU associated with limited therapeutic options (Vardakas et al. 2015).

Herein, we report an investigation of CRKP carriage and acquisition in the ICU to illustrate the clonal spread of CRKP isolates, their phenotypic and genotypic characteristics, and to track their evolutionary traits further.

**Experimental**

**Materials and Methods**

**CRKP isolates and Antimicrobial Susceptibility Testing.** All the carbapenem-resistant *K. pneumoniae* strains were isolated from clinical specimens of the ICU patients in our hospital (Hwa Mei Hospital, University of Chinese Academy of Sciences, Ningbo, China) between October 2016 and March 2019. The identification of these isolates and antimicrobial susceptibility testing were done using the VITEK 2 Compact automated system (BioMérieux, Marcy l’Etoile, France). The routine antibiotic panel comprised ertapenem, amoxicillin/clavulanic acid, amikacin, aztreonam, ciprofloxacin, ceftiraxone, cefazolin, nitrofurantoin, cefepime, ceftoxitin, gentamicin, imipenem, levofloxacin, trimethoprim/sulfamethoxazole, tobramycin, piperacillin/tazobactam, ampicillin, and tigecycline. The susceptibilities to ertapenem, imipenem, and tigecycline were confirmed by the disk diffusion method or E-test. According to manufacturer’s instructions, *Enterobacter hormaechei* ATCC 700323 and *Escherichia coli* ATCC 25922 were used as controls for species identification and susceptibility testing, respectively. The isolates resistant to either ertapenem or imipenem were defined as CRKP isolates in this study. Antimicrobial susceptibility results were interpreted by the criteria of the Clinical and Laboratory Standards Institute (CLSI 2018). Patient clinical information was acquired from electronic medical records, and the Ethics Committee of our hospital approved the study.

**Detection of resistance determinants.** All the isolates studied were tested for the production of carbapenemases by the modified carbapenem inactivation method (mCIM) recommended by CLSI (CLSI 2018). The genes encoding carbapenemases were investigated by polymerase chain reaction (PCR) using a series of primers as previously reported (Queenan and Bush 2007; Nordmann et al. 2011). The amplification products were sent for DNA sequencing (Qingke BioTech, Hangzhou).

**Isolates genotyping.** CRKP isolates in the study were genotyped by multilocus sequence typing (MLST). Seven housekeeping genes including gapA, infB, mdh, pgi, phoE, rpoB, and tonB of *K. pneumoniae* were amplified and sequenced based on protocols as described (Diancourt et al. 2005). Sequence types (STs) were identified using the online database at the Pasteur Institute multilocus sequence typing website for *K. pneumoniae* (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). The evolutionary relationships between isolates were analyzed by the platform-independent Java software PHYLOVIZ using the goeBURST algorithm at a single-locus variant (SLV) level.

**Results**

**Demographic and clinical characteristics of CRKP carriers.** A total of 30 CRKP strains were isolated from 26 patients in the intensive care unit (ICU), and the strains from different isolation sites of the same patient were included in this study. Among these CRKP isolates, 12 (40%) were isolated from sputum specimens, and the remaining isolates were obtained from other types of specimens including blood (three, 10%), wound (two, 6.7%), drainage fluid (four, 13.3%), and bronchial perfusate (one, 3.3%). 69.2% (18) of the patients were male, and 76.9% (20) were over 60 years old.

All patients had undergone invasive procedures such as tracheal intubation and central venous catheterization. During the ICU admission, multiple antimicrobials were used for the treatment of various intercurrent infections. Among the 26 patients with CRKP acquisition, five died, six declined further therapy, and 15 were discharged from the hospital ward. The times from acquisition of CRKP to death for the five patients who died, listed in order, were four, 107, 29, 36, and 16 days, respectively. Other detailed records of patients and information on the bacteria were summarized in Table I.
Table I  The corresponding bacterial characteristics and medical records of patients with CRKP acquisition.

| Bacterial strain | Age | Isolation site | ST | Carbapenemases | Underlying conditions                                                                 | Invasive procedures                                      | Antimicrobial treatment | The length of stay | Outcomes                |
|------------------|-----|----------------|----|----------------|----------------------------------------------------------------------------------------|----------------------------------------------------------|------------------------|---------------------|----------------------|
| 1025             | 61  | Wound          | NA | NDM-5          | Septic shock, MODS, necrotizing fasciitis                                               | Nasogastric tube, central venous catheter, surgical procedure | IMP, AMC, FEP, MFX, ISE | 23 days            | Survived            |
| 1029             | 81  | Wound          | ST722 | NA           | Pulmonary infection, hypertension, diabetes mellitus, Pulmonary failure                 | PICC catheter, mechanical ventilation heart disease, encephalorrhagia | IMP, TZP, SCF         | 46 days            | Stable, discharged   |
| 1050             | 73  | Blood          | ST11 | NDM-5         | Septic shock, biliary tract infection, COPD, MODS, Pulmonary failure                    | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, IMP, MEM, SCF, TGC | 55 days            | Discontinuing treatment |
| 1051             | 89  | Sputum         | ST11 | NDM-5         | Pulmonary infection, septic shock, pulmonary failure, Hypertension, chronic renal failure, MDS, diabetes mellitus, hypertensive heart disease | Mechanical ventilation, urinary catheter, gastric tube | SCF, TGC              | 20 days            | Death               |
| 1052             | 73  | Sputum         | ST11 | NDM-5         | Septic shock, biliary tract infection, COPD, MODS, Pulmonary failure                    | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, IMP, MEM, SCF, TGC | 34 days            | Discontinuing treatment |
| 1055             | 46  | Drainage fluid | ST1446 | NA            | Pulmonary infection, pyothorax, septic shock, Pulmonary failure, renal insufficiency, liver cirrhosis | Mechanical ventilation, urinary catheter, deep vein catheter, gastric tube | IMP, TZP, SCF         | 38 days            | Discontinuing treatment |
| 1062             | 79  | Bronchial perfusate | ST290 | NDM-5       | Pulmonary infection, chronic bronchitis, Pulmonary failure, hypertension, cerebral infarction, pleural effusion | Mechanical ventilation, urinary catheter, deep vein catheter, gastric tube | SCF, AMC, FEP       | 26 days            | Survived            |
| 1063             | 79  | Sputum         | ST290 | NDM-5         | Pulmonary infection, chronic bronchitis, Pulmonary failure, hypertension, cerebral infarction, pleural effusion | Mechanical ventilation, urinary catheter, deep vein catheter, gastric tube | SCF, AMC, FEP       | 26 days            | Survived            |
| 1064             | 61  | Urine          | ST290 | NDM-5         | Spinal cord injury, high falling injury, Electric injury, Pulmonary infection, Pulmonary failure, fracture | Mechanical ventilation, urinary catheter, deep vein catheter, gastric tube | TZP, MEM, SCF        | 83 days            | Stable, discharged   |
| 1076             | 42  | Sputum         | ST290 | NDM-5         | Cranioencebral trauma, Pulmonary contusion, Hemorrhagic shock, deep venous thrombosis, Renal failure | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, AMC, FEP, ISE, MEM, SCF | 136 days          | Death               |
| 1102             | 79  | Sputum         | ST290 | NDM-5         | Pulmonary infection, Pulmonary failure, Chronic bronchitis, Parkinson                     | Mechanical ventilation, urinary catheter, deep vein catheter, gastric tube, surgical procedure | SCF, IMP, TZP        | 123 days           | Survived            |
| 1165             | 76  | Drainage fluid | ST11 | KPC           | Gastric perforation, Peritonitis, Fistula of colon, Pulmonary infection, Liver cirrhosis | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, IMP, AMC, FEP, TGC, SCF | 18 days            | Discontinuing treatment |
| 1233             | 38  | Drainage fluid | ST290 | NA           | Retroperitoneal abscess, Acute necrotizing pancreatitis, Hepatic insufficiency, Hyperlipemia | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, LEV, IMP        | 46 days            | Discontinuing treatment |
| 1247             | 72  | Urinary catheter | ST11 | KPC          | NMS, Pulmonary infection, Pulmonary failure, Diabetes mellitus, Hypertension, Renal or Hepatic insufficiency, Hyperlipemia | Mechanical ventilation, urinary catheter, deep vein catheter | TZP                  | 27 days            | Stable, discharged   |
| 1762             | 57  | Sputum         | ST11 | KPC           | Septic shock, Pulmonary encephalopathy, COPD, Pulmonary failure, Hypertension, Fungal infection | Mechanical ventilation, urinary catheter, deep vein catheter | SCF, IMP, AMK, FEP   | 45 days            | Death               |
| 1773             | 88  | Urinary catheter | ST11 | NA           | Pulmonary infection, Pulmonary failure, Cerebral infarction, Hypertension, Diabetes mellitus, Alzheimer Disease | Mechanical ventilation, urinary catheter, gastric tube | TZP, IMP              | 35 days            | Stable, discharged   |
### Table I continued.

| Bacterial strain | Age | Isolation site | ST | Carbapenemases | Underlying conditions | Invasive procedures | Antimicrobial treatment | The length of stay | Outcomes |
|------------------|-----|----------------|----|----------------|-----------------------|---------------------|------------------------|---------------------|----------|
| Urinary catheter | 39  | NA             | ST111 | NA            | MODS, pulmonary failure, traumatic shock, multiple fracture, sepsis, pulmonary infection, fungal infection | Mechanical ventilation, urinary catheter, deep vein catheter | IMP, SCF, ISE, MXF | 183 days | Survived |
| Urinary catheter | 72  | NA             | ST11 | KPC           | SCAP, pulmonary failure, urinary tract infection, diabetes mellitus, septic shock, fungal infection, pleural effusion | Mechanical ventilation, urinary catheter, deep vein catheter | IMP, SCF, TGC, AZM, MXF | 31 days | Discontinuing treatment |
| Urinary catheter | 70  | NA             | NA   | NA            | Pulmonary infection, pulmonary failure, septic shock, fungal infection, cerebral infarction, hypertension, diabetes mellitus, gastrointestinal hemorrhage | Mechanical ventilation, urinary catheter, deep vein catheter, gastrointestinal tube | TZP, SCF, CIP, FEP | 40 days | Survived |
| Sputum           | 75  | ST111          | NA   | KPC           | Lung cancer, pulmonary infection, bronchiectasis, CHD, hypertension, diabetes mellitus | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, SCF, IMP, TGC | 87 days | Survived |
| Sputum           | 78  | ST896          | KPC  | SCAP          | Cerebral aneurysm, subarachnoid hemorrhage, intracranial infection, pulmonary infection, deep venous thrombosis, fungal infection | Mechanical ventilation, urinary catheter | TZP, MEM, LEV, ISE, IMP, PB | 63 days | Stable, discharged |
| Sputum           | 85  | ST11           | KPC  | SCAP          | Pulmonary infection, pulmonary failure, cardiac failure, cerebral infarction, hypertension | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | IMP, TZP, TGC | 66 days | Stable, discharged |
| Sputum           | 78  | ST11           | KPC  | SCAP          | Pulmonary infection, severe pneumonia, pulmonary failure, renal failure, pulmonary arterial hypertension | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | IMP, SCF, TGG, PB, CAZ/AVI | 46 days | Death |
| Sputum           | 82  | ST11           | KPC  | SCAP          | Pulmonary infection, severe pneumonia, pulmonary failure, renal failure, hypertension, hepatic insufficiency, gastrointestinal hemorrhage, deep venous thrombosis | Mechanical ventilation, urinary catheter, deep vein catheter | SCE, MXF, MEM, IMP, TGC, AMK, PB, CAZ/AVI, MH | 51 days | Discontinuing treatment |
| Sputum           | 82  | ST11           | KPC  | SCAP          | Tonsil carcinoma, hypertension, hyperlipemia, interstitial pneumonia, pulmonary failure, fungal infection | Mechanical ventilation, urinary catheter | IMP | 47 days | Stable, discharged |
| Sputum           | 80  | ST11           | KPC  | SCAP          | COPD, pulmonary failure, pulmonary encephalopathy, cardiac failure, renal failure, pulmonary arterial hypertension | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | IMP | 47 days | Stable, discharged |
| Sputum           | 82  | ST11           | KPC  | SCAP          | COPD, pulmonary failure, pulmonary encephalopathy, diabetes mellitus, hypertension, fracture, septic shock, gastrointestinal hemorrhage | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | TZP, IMP, SCF | 37 days | Death |
| Drainage fluid   | 66  | NA             | ST11 | NDM-1, KPC    | Peritonitis, septic shock, pulmonary failure, renal failure, hypertension, fungal infection, intestinal perforation | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter, drainage tube | IMP, TGC, TZP, SCF | 43 days | Stable, discharged |
| Urinary catheter | 89  | ST111          | KPC  | SCAP          | Severe pneumonia, pulmonary failure, CHD, hypertension, rectal cancer, myocardial infarction, fungal infection | Mechanical ventilation, urinary catheter, deep vein catheter | TZP, MEM, SCF, PB, TGC, CAZ/AVI | 22 days | Survived |
| Blood            | 85  | ST11           | KPC  | SCAP          | COPD, pulmonary failure, pulmonary encephalopathy, cardiac failure, renal failure, pulmonary arterial hypertension | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | IMP, SCF, TGC, PB, CAZ/AVI | 46 days | Death |
| Blood            | 82  | ST11           | KPC  | SCAP          | COPD, pulmonary failure, pulmonary encephalopathy, diabetes mellitus, hypertension, fracture, septic shock, gastrointestinal hemorrhage | Mechanical ventilation, urinary catheter, deep vein catheter, PICC catheter | TZP, IMP, SCF | 37 days | Death |

**Legends:**
- MODS – Multiple organ dysfunction syndrome
- COPD – Chronic obstructive pulmonary disease
- MDS – Myelodysplastic syndrome
- NMS – Neurologic malignant syndrome
- CHD – Coronary heart disease
- IMP – Imipenem
- AMC – Amoxicillin/clavulanic acid
- FEP – Cefepime
- MXF – Moxifloxacin
- ISE – Isoniazid
- TZP – Piperacillin/tazobactam
- SCF – Cefperazone/sulbactam
- MEM – Meropenem
- TGC – Tigecycline
- LEV – Levofloxacin
- AMK – Amikacin
- CIP – Ciprofloxacin
- PB – Polymyxin B
- CAZ/AVI – Ceftazidime/avibactam
- MH – Minocycline
- ST – Sequence type
- NA – Not available
Antimicrobial susceptibility. The isolates in this study were resistant to nearly all clinically available antimicrobials; more than half of the isolates were only susceptible to one or two kinds of antimicrobials and were called extensive drug-resistant isolates. All isolates presented resistance to ertapenem, amoxicillin/clavulanic acid, cefoperazone/sulbactam, cefazolin, cefoxitin, and ampicillin. The isolates were relatively susceptible to four antimicrobials: gentamicin, tigecycline, tobramycin, and amikacin, to which the resistance rates were 36.7, 30.0, 26.7, and 16.7%, respectively. The percentage of resistant isolates to each antibiotic was shown in Fig. 1.

Profiling of resistance determinants. The majority of 30 CRKP (n = 23, 76.7%) isolates were positive for the mCIM test. The results of PCR and DNA sequencing showed that nine isolates (30%) harbored the blaNDM-5 gene, 12 isolates 40% harbored the blaKPC-2 gene, and one isolate had the blaNDM-1 gene. The coexistence of blaNDM-1 and blaKPC-2 in one isolate was also noticed. The carbapenemase-encoding genes were not detectable in seven isolates.

Bacterial clonal relatedness. Among 30 CRKP isolates, six sequence types (STs) were detected, namely ST722, ST1446, ST111, ST896, ST290, and ST11 as shown by MLST. ST290 and ST11 accounted for 20% (6/30) and 56.7% (17/30) of all isolates tested, whereas the other STs were sporadic. Three ST11 isolates carried blaNDM-5 yet blaKPC-2 was more likely to be identified among ST11 clones. By contrast, ST290 clones harbored only blaNDM-5. Seven isolates that were negative for mCIM test belonged to diverse STs (ST722, ST1446, ST290, ST11, and ST111). Notably, two K. pneumoniae NDM-producers failed to be classified into any sequence types and there was the sole isolate that was negative for either mCIM or sequence typing. Figure 2 displays the annotated minimum spanning tree showing that ST1446, ST111, ST896, ST290, and ST111 clones belonged to different groups, except for ST722 listed in the sub-group of ST11 group.

Discussion

In 2017, WHO published a global priority pathogens list of antibiotic-resistant bacteria, in which CRE was ranked among the Priority 1 pathogens (WHO 2017). Carbapenem-resistant K. pneumoniae, which is the most common carbapenem-resistant Enterobacteriaceae (CRE), has already generated a worrisome crisis of epidemiological, clinical, and infection control issues worldwide (Bradford et al. 2004; Maltezou et al. 2009),
including the ICUs across China (Zhang et al. 2011; Yu et al. 2012; Yu et al. 2019).

In this study, we have described the polyclonal spread of CRKP isolates of six distinct sequence types (ST290, ST11, ST722, ST1446, ST111, and ST896) in the same ward (ICU). Among these isolates, two novel clones of ST722 and ST1446 were found, and they did not produce carbapenemases since the negative results of the mCIM test were obtained. It inferred that other mechanisms of resistance might be relevant such as hyperproduction of ESBL enzymes, AmpC β-lactamases, or alteration of outer membrane porins as well as regulation of efflux systems (Kaczmarek et al. 2006; Bush and Jacoby 2010; Filgona et al. 2015). The minimum spanning tree demonstrated that ST722 clone probably shared the same ancestor with ST11 clone in the evolutionary process. As for ST111, it has long been identified among carbapenem-resistant, and ESBL-producing *K. pneumoniae* isolates, e.g., obtained from Riyadh (uz Zaman et al. 2014), South India (Kumar et al. 2018), New York (Diago-Navarro et al. 2014), and New Zealand (Lester et al. 2011). Further, the ST11 *K. pneumoniae*, one type of clone responsible for the outbreak of multi-drug carbapenem-resistant *K. pneumoniae* in Riyadh, carried the OXA-48 gene, suggesting that the acquisition of carbapenem-resistance genes by *K. pneumoniae* of different STs may contribute to the emergence of diverse CRKP clones. By contrast, only one ST896 CRKP isolate that was identified from Heilongjiang Province in China harbored the *bla*<sub>IMP-4</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>TEM</sub> genes (Gong et al. 2018).

Therefore, this is also the first report on the observation of the *bla*<sub>KPC-2</sub>-harboring ST896 CRKP clone in China.
The emergence of sporadic clones producing MBLs, e.g., producing NDM-5 CRKP isolates of ST290 and ST11 clones, is a warning signal of the genotypic switch in epidemic KPC-producing CRKP population. Therefore, valid interventions should be developed to avoid outbreaks caused by novel subclones.

Acknowledgments

The authors thank their colleagues from the first affiliated hospital of Wenzhou Medical University for participating in the research and the authors also express their gratitude to the editors of this Journal for suggesting reviewers and commenting. This work was supported by Ningbo Natural Science Foundation, China (Grant No. 2018A610399).

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Bradford PA, Bratu S, Urban C, Visalli M, Mariano N, Landman D, Rahal JJ, Brooks S, Cebular S, Quale J. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamas in New York City. *Clin Infect Dis.* 2004 Jul 01;39(1):55–60. https://doi.org/10.1086/421495

Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, Quale J. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. *Arch Intern Med.* 2005 Jun 27;165(12):1430–1435. https://doi.org/10.1001/archinte.165.12.1430

Bush K, Jacoby GA. Updated functional classification of beta-lactamas. *Antimicrob Agents Chemother.* 2010 Mar;54(3):969–976. https://doi.org/10.1128/AAC.01009-09

CLSI. *Performance standards for antimicrobial susceptibility testing*. 28th Informational Supplement (M100-S28). Wayne (USA): Clinical and Laboratory Standards Institute; 2018.

Debby BD, Ganor O, Yasmin M, David L, Nathan K, Ilana T, Dalit S, Smollan G, Galia R. Epidemiology of carbapenem resistant *Klebsiella pneumoniae* colonization in an intensive care unit. *Eur J Clin Microbiol Infect Dis.* 2012 Aug;31(8):1811–1817. https://doi.org/10.1007/s10096-011-1506-5

Diago-Navarro E, Chen I, Passet V, Burack S, Ulacia-Hernando A, Kodyanplakkal RP, Levi MH, Brisse S, Kreiswirth BN, Fries BC. Carbapenem-resistant *Klebsiella pneumoniae* exhibit...
variability in capsular polysaccharide and capsule associated virulence traits. J Infect Dis. 2014 Sep 01;210(5):803–813. https://doi.org/10.1093/infdis/jiu157

Diancourt L, Passet V, Verhoef J, Grimont PAD, Brisse S. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J Clin Microbiol. 2005 Aug 01;43(8):4178–4182. https://doi.org/10.1128/JCM.43.8.4178-4182.2005

Filgona J, Banerjee T, Anupurba S. Role of efflux pumps inhibitor in decreasing antibiotic resistance of Klebsiella pneumoniae in a tertiary hospital in North India. J Infect Dev Ctries. 2015 Aug 29(9(08)):815–820. https://doi.org/10.3855/jjdc.6216

Gong X, Zhang J, Su S, Fu Y, M. Wang Y, Zhang X. Molecular characterization and epidemiology of carbapenem non-susceptible Enterobacteriaceae isolated from the Eastern region of Heilongjiang Province, China. BMC Infect Dis. 2018 Dec 18(1):417. https://doi.org/10.1186/s12879-018-3294-3

Hammerum AM, Hansen F, Lester CH, Jensen KT, Hansen DS, Dessau RB. Detection of the first two Klebsiella pneumoniae isolates with sequence type 258 producing KPC-2 carbapenemase in Denmark. Int J Antimicrob Agents. 2010 Jun;35(6):610–612. https://doi.org/10.1016/j.ijantimicag.2010.01.024

Hu L, Liu Y, Deng I, Zhong Q, Hang Y, Wang Z, Zhan I, Wang L, Yu F. Outbreak by ventilator-associated ST11 K. pneumoniae with co-production of CTX-M-24 and KPC-2 in a SICU of a tertiary teaching hospital in central China. Front Microbiol. 2016 Aug 02;7:1190. https://doi.org/10.3389/fmicb.2016.01190

Hussein K, Sprecher H, Mishachi T, Oren I, Kassis I, Finkelstein R. Carbapenem resistance among Klebsiella pneumoniae isolates: risk factors, molecular characteristics, and susceptibility patterns. Infect Control Hosp Epidemiol. 2009 Jul;30(7):666–671. https://doi.org/10.1086/598244

Ji S, Lv F, Du X, Wei Z, Fu Y, Mu X, Jiang Y, Yu Y. Ceftazime combined with amoxicillin/clavulanic acid: a new choice for the KPC-producing K. pneumoniae infection. Int J Infect Dis. 2015 Sep;38:108–114. https://doi.org/10.1016/j.ijid.2015.07.024

Kaczmarek FM, Dynaki F, Baskaran A, Paul M, Ponnudh S, Santaham S, Michael J, Veeraraghavan B. Molecular characterisation for clonality and transmission dynamics of an outbreak of Klebsiella pneumoniae amongst neonates in a tertiary care centre in South India. Indian J Med Microbiol. 2018;36(1):54–60. https://doi.org/10.4103/immm.IJMM_17_42

Lester CH, Olsen SS, Jakobsen L, Arpi M, Fuursted K, Hansen DS, Helberg O, Holm A, Heijbjerg T, Jensen KT, et al. Emergence of extended-spectrum β-lactamase (ESBL)-producing Klebsiella pneumoniae in Danish hospitals; this is in part explained by spread of two CTX-M-15 clones with multilocus sequence types 15 and 16 in Zealand. Int J Antimicrob Agents. 2011 Aug;38(2):180–182. https://doi.org/10.1016/j.ijantimicag.2011.03.018

Logan IK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: The impact and evolution of a global menace. J Infect Dis. 2017. 215(suppl_1):S28–S36.

Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, Lynfield R, Maloney M, McAllister-Holod L, Nadle J, et al.; Emerging Infections Program Healthcare-Associated Infections and Antimicrobial Use Prevalence Survey Team. Multistate point-prevalence survey of health care-associated infections. N Engl J Med. 2014 Mar 27;370(11):1198–1208. https://doi.org/10.1056/NEJMoa1306801

Maltezou HC, Giakoupi P, Maragos M, Bolikas M, Raftopoulos V, Papahatzaki H, Viouhos G, Liakou V, Vatopoulos AC. Outbreak of infections due to KPC-2-producing Klebsiella pneumoniae in a hospital in Crete (Greece). J Infect. 2009 Mar;58(3):213–219. https://doi.org/10.1016/j.jinf.2009.01.010

Martin RM, Bachman MA. Colonization, infection, and the access genome of Klebsiella pneumoniae. Front Cell Infect Microbiol. 2018 Jan 22;8:4. https://doi.org/10.3389/fcimb.2018.00004

Mathers AJ, Peirano G, Pitout JDD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clin Microbiol Rev. 2015 Jul;28(3):565–591. https://doi.org/10.1128/CMR.00116-14

Nordmann P, Poirot L, Carrè r A, Toleman MA, Walsh TR. How to detect NDM-1 producers. J Clin Microbiol. 2011 Feb 01;49(2):718–721. https://doi.org/10.1128/JCM.01773-10

Papadimitr ou-Olvigeris M, Marangos M, Filigou F, Christofidou M, Sklavou C, Vamvakopoulou S, Anastassiou ED, Filos KS. KPC-producing Klebsiella pneumoniae enteric colonization acquired during intensive care unit stay: the significance of risk factors for its development and its impact on mortality. Diagn Microbiol Infect Dis. 2013 Oct;77(2):169–173. https://doi.org/10.1016/j.diagmicrobio.2013.06.007

Qi Y, Wei Z, Ji S, Du X, Shen P, Yu Y, ST11, the dominant clone of KPC-producing Klebsiella pneumoniae in China. J Antimicrob Chemotherapy. 2011 Feb;66(2):307–312. https://doi.org/10.1093/jac/dkq431

Queenan AM, Bush K. Carbapenemases: the versatile β-Lactamases. Clin Microbiol Rev. 2007 Jul;20(3):440–458. https://doi.org/10.1128/CMR.00001-07

Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant Klebsiella pneumoniae acquisition among hospitalized adults and effect of acquisition on mortality. Antimicrob Agents Chemother. 2008 May;52(5):1028–1033. https://doi.org/10.1128/AAC.01020-07

Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Henderson DK, Palmore TN, Segre JA; NISC Comparative Sequencing Program Group. Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae. J Clin Microbiol. 2011 May;49(5):1508–1512. https://doi.org/10.1128/JCM.00116-14

Tian L, Zhang Z, Sun Z. Antimicrobial resistance trends in bloodstream infections at a large teaching hospital in China: a 20-year surveillance study (1998–2017). Antimicrob Resist Infect Control. 2019 Dec;8(1):86. https://doi.org/10.1186/s13756-019-0455-z

Uz Zaman T, Aldrees M, Al Johani SM, Alrodayah M, Aldughashem FA, Balkhy HH. Multi-drug carbapenem-resistant Klebsiella pneumoniae infection carrying the OXA-48 gene and showing variations in outer membrane protein 36 causing an outbreak in a tertiary care hospital in Riyadh, Saudi Arabia. Int J Infect Dis. 2014 Nov;28:186–192. https://doi.org/10.1016/j.ijid.2014.05.021
Polyclonal spread of CRKP isolates in ICU

Vardakas KZ, Matthaiou DK, Falagas ME, Antypa E, Koteli A, Antoniadou E. Characteristics, risk factors and outcomes of carbapenem-resistant Klebsiella pneumoniae infections in the intensive care unit. J Infect. 2015 Jun;70(6):592–599. https://doi.org/10.1016/j.jinf.2014.11.003

Wang Z, Li M, Shen X, Wang L, Liu L, Hao Z, Duan J, Yu F. Outbreak of bla<sub>NDM-5</sub>-Harboring Klebsiella pneumoniae ST290 in a Tertiary Hospital in China. Microb Drug Resist. 2019 Dec 01;25(10):1443–1448. https://doi.org/10.1089/mdr.2019.0046

WHO. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics [Internet]. Geneva (Switzerland): World Health Organization; 2017 [cited 2017 February 27]. Available from https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/

Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. NDM metallo-β-Lactamases and their bacterial producers in health care settings. Clin Microbiol Rev. 2019 Jan 30;32(2):e00115-18. https://doi.org/10.1128/CMR.00115-18

Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR. Characterization of a new metallo-β-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother. 2009 Dec;53(12):5046–5054. https://doi.org/10.1128/AAC.00774-09

Yu F, Hu L, Zhong Q, Hang Y, Liu Y, Hu X, Ding H, Chen Y, Xu X, Fang X, et al. Dissemination of Klebsiella pneumoniae ST11 isolates with carbapenem resistance in integrated and emergency intensive care units in a Chinese tertiary hospital. J Med Microbiol. 2019 Jun 01;68(6):882–889. https://doi.org/10.1099/jmm.0.000981

Yu F, Ying Q, Chen C, Li T, Ding B, Liu Y, Lu Y, Qin Z, Parsons C, Salgado C, et al. Outbreak of pulmonary infection caused by Klebsiella pneumoniae isolates harbouring bla<sub>IMP</sub> and bla<sub>NDM-1</sub> in a neonatal intensive care unit in China. J Med Microbiol. 2012 Jul 01;61(7):984–989. https://doi.org/10.1099/jmm.0.043000-0

Zhang P, Shi Q, Hu H, Hong B, Wu X, Du X, Akova M, Yu Y. Emergence of ceftazidime/avibactam resistance in carbapenem-resistant Klebsiella pneumoniae in China. Clin Microbiol Infect. 2020 Jan;26(1):e1-124.e4. https://doi.org/10.1016/j.cmi.2019.08.020

Zhang R, Wang XD, Cai JC, Zhou HW, LvHX, Hu QF, Chen GX. Outbreak of Klebsiella pneumoniae carbapenemase 2-producing K. pneumoniae with high qnr prevalence in a Chinese hospital. J Med Microbiol. 2011 Jul 01;60(7):977–982. https://doi.org/10.1099/jmm.0.015826-0