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Epidemiology of resistance of carbapenemase-producing *Klebsiella pneumoniae* to ceftazidime-avibactam in a Chinese hospital

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Abstract

Aims: *Klebsiella pneumoniae* has been reported to develop increased antibiotic resistance. Ceftazidime-avibactam (CZA) is a novel antibiotic with activity against serine-lactamase. Here, we investigated the sensitivity of carbapenem-resistant *K. pneumoniae* (CRKP) to CZA and the mechanisms of drug resistance in our hospital.

Methods and Results: Patient characteristics were obtained from medical records. *K. pneumoniae* and its antibiotic susceptibility were determined using the Vitek-2 Compact instrument. The antibiotic resistance genes *KPC*, *NDM*, *OXA-48*, *VIM*, *IMP*, *CIM*, *SPM*, *TMB*, *SMB*, *SIM*, *AIM* and *DIM* were detected using real-time PCR. Multilocus sequence typing was used for genetic RELATEDNESS analysis. In total, 121 CRKP strains were isolated from patients in the intensive care unit (51·2%), senior ward (12·4%) and neurosurgery department (10%). With an average age of 72·5 years, most patients were in care for respiratory (34·7%), brain (20·7%), digestive tract (13·2%) and cardiovascular (8·3%) diseases. Specimens were predominantly obtained from sputum (39·67%), urine (29·75%) and blood (6·61%).

Conclusion: Of 23 CZA-resistant CRKP strains (19·01%), ST11 being the most common at 56·52%, 11 *NDM-1*-positive (47·83%) and four *NDM-5*-positive (17·39%) strains were detected.

Significance and Impact of the Study: Our study indicates that CZA resistance occurs in ~19·01% CRKP strains and that bla_{NDM-1} and bla_{NDM-5} might be critical for resistance.

KEYWORDS

carbapenem resistance, ceftazidime-avibactam, epidemiology, *Klebsiella pneumoniae*, NDM-1, NDM-5

INTRODUCTION

In recent years, *Klebsiella pneumoniae* has become the second most common Gram-negative pathogenic bacteria in China after *Escherichia coli*. Carbapenems are the last resort drugs for treating infections caused by multidrug-resistant Gram-negative bacteria. However, carbapenem-resistant pathogens have emerged with the increasing clinical use of

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carbapenems and now pose a great threat to human health. The current selection of antibiotics for the treatment of carbapenem-resistant Enterobacteriaceae (CRE) infections, including polymyxin, tigecycline, fosfomycin and aminoglycoside, is limited. Therefore, new and effective anti-CRE therapies are urgently needed. Ceftazidime-avibactam (CZA) was developed by AstraZeneca Pharmaceuticals Co., Ltd. and was approved by the United States Food and Drug Administration (FDA) in February 2015 (Mendes *et al.* 2015). It combines ceftazidime with a non-β-lactam β-lactamase inhibitor, avibactam, and is used to treat complex abdominal and urinary tract infections, hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia (Stone *et al.* 2017). CZA is effective against most Gram-negative bacteria but is only recommended for use against infections caused by pathogens that exhibit severe drug resistance (Carmeli *et al.* 2016). This is because, as with all drugs, bacteria can develop resistance to these novel inhibitors. In the current study, the mechanism of CZA resistance in carbapenemase-producing *K. pneumoniae* was investigated with the intention of providing a molecular biological basis for the clinical treatment of carbapenem-resistant *K. pneumoniae* (CRKP).

MATERIALS AND METHODS

Characterization of Patients and CRKP

Patient characteristics, including age, sex, inpatient ward and disease symptoms, were obtained from electronic medical records between January 2017 and June 2019. *Klebsiella pneumonia*, as well as antibiotic susceptibility, were determined using the Vitek-2 Compact instrument (bioMérieux, Marcy-l'Étoile, France). This study was approved by the ethics committee of the hospital, the ethics approval number is K2018-01-001.

CZA Susceptibility

Drug sensitivity was determined by microbroth dilution. CZA (Lot: DZ0202) and cation-adjusted Mueller Hinton broth (CAMHB; Lot: hb6231-1) were purchased from Bio-Kont (Wenzhou, China). The concentration of CZA was increased from 0·06/4 to 64/4 µg ml^{-1}. Added 10-µl bacterial suspension (0·5 MC) into 2-ml CAMHB, blended them and then 100 µl was added to each well. The drug sensitivity plate was placed in a 5% carbon dioxide incubator overnight, and the results were observed the next day. To establish the minimum inhibitory concentration (MIC) of CZA for each bacterial strain, the CLSI M100-S27 standard was used. Strains with a CZA MIC ≤ 8/4 µg ml^{-1} were considered sensitive, whereas strains with a CZA MIC ≥ 16/4 µg ml^{-1} were considered resistant.

Multilocus Sequence Typing

Bacterial DNA was extracted using the Bacterial Genomic DNA Kit (CWBIO Co., Ltd, Beijing, China). Multilocus sequence typing (MLST) for *K. pneumoniae* was performed using the methods described previously (Diancourt *et al.* 2005). The allelic profiles and sequence types (STs) were determined using online databases (https://pubmlst.org/bigsdb?db=pubmlst_mlst_seqdef). All ST sequences longer than 3012 bp were spliced and aligned with the accepted sequences downloaded from the PubMLST website. Then, a phylogenetic tree was constructed with MEGA (https://megasoftware.net/) using a 10 algorithm.

Detection of Antibiotic Resistance Genes

The primers for the amplification of resistance genes (*KPC*, *NDM*, *OXA-48*, *VIM*, *IMP*, *CIM*, *SPM*, *TMB*, *SMB*, *SIM*, *AIM* and *DIM*) were designed by Primer Premier 5 (ver. 5.0). Bacterial DNA was extracted as described above. Quantitative polymerase chain reaction (real-time PCR) was performed with an UltraSYBR mixture kit (CWBIO Co., Ltd) on a Cobas z 480 analyser (LightCycler 480; Roche, Basel, Switzerland) with an initial incubation at 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. Melting curve fluorescence was evaluated five times per degree Celsius from 60 to 95°C. Each reaction was carried out in triplicate.
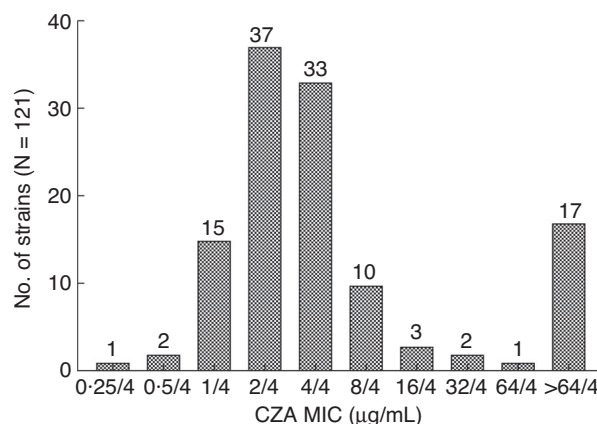
RESULTS

Results of the Characterization of Patients and CRKP

We isolated 121 CRKP strains from our hospital between January 2017 and June 2019. The characteristics of patients and bacterial strains are shown in Table 1. The age of the patients ranged from 6 months to 98 years, with an average age of 72·54. Furthermore, 76·86% of patients were over the age of 60, and males outnumbered females 2·27 to 1 (84/37). The specimens were from various sources, such as sputum, urine, blood, bronchial secretions, perianal swabs, pleural effusion, wound secretions, ascites and bile. The three most common sources for the specimens were sputum (39·67%), urine (29·75%) and blood (6·61%). The patients were diagnosed with a variety of conditions, including pulmonary infection, stroke, acute pancreatitis, bile duct stones, urinary

TABLE 1 Demographic and clinical characteristics of patients and specimens ($N = 121$)

Characteristics (unit)	Numerical data, n (%)
Median age (years)	76
Interquartile range (years)	25
Categories by age (years), N (%)	
<20	1 (0·8)
20–40	9 (7·4)
41–60	18 (14·9)
61–80	37 (30·6)
81–100	56 (46·3)
Gender, no. of males (%)	84 (69·4)
Source, N (%)	
Sputum	48 (39·7)
Urine	36 (29·7)
Blood	8 (6·6)
Others	29 (24·0)
Comorbidities, N (%)	
Pulmonary infections	42 (34·7)
Brain diseases	25 (20·7)
Diseases of digestive system	16 (13·2)
Heart diseases	10 (8·3)
Diseases of urinary system	7 (5·8)
Other	21 (17·3)
Origin, N (%)	
Intensive care unit	62 (51·2)
Senior officials inpatient ward	15 (12·4)
Neurosurgery	12 (10·0)
Hepatobiliary surgical ward	4 (3·3)
Others	28 (23·1)
Average hospital stay (days)	49
Interquartile range (days)	21–56
Outcomes, N (%)	
Crude 7-day mortality	2 (1·7)
Crude 30-day mortality	7 (5·8)
Total mortality	9 (7·4)

FIGURE 1 Minimum inhibitory concentrations (MICs) of ceftazidime-avibactam. Concentrations of ceftazidime-avibactam ranging between 0·25/4 and 128/4 µg ml^{-1} were used to determine the MICs of this combination of antimicrobials using 121 isolated carbapenem-resistant *Klebsiella pneumoniae* strains.

tract infection and bloodstream infections. The four most common conditions were respiratory disease (34·71%), brain disease (20·66%), digestive tract disease (13·22%) and cardiovascular disease (8·26%). Patients were located in many wards, including the intensive care unit (ICU), respiratory department, gastroenterology department, haematology department, thoracic surgery department, emergency department, cardiovascular department, traditional Chinese medicine department, orthopaedic burn department, neurosurgery department, hepatobiliary surgery department, rehabilitation department and the senior official inpatient ward. The patients were most commonly located in the ICU (51·24%), senior ward (12·40%), neurosurgery department (9·92%) and hepatobiliary surgery department (3·31%). The outcomes of the CRKP-infected patients were crude 7-day mortality of 1·7% (two patients), crude 30-day mortality of 5·8% (seven patients) and total mortality of 7·4% (nine patients).

MIC and Antibiotic Efficacy of CZA Against CRKP

The rate of resistance of 121 strains of CRKP against CZA was 19·01% (23/121). The MIC of CZA for each strain is shown in Fig. 1.

Sequence Typing and Phylogenetic Tree Analysis

For each CRKP strain, genomic DNA was extracted, and allelic profiling was performed. DNA sequencing results were compared and analysed on an MLST database, and eight STs (ST11, ST147, ST859, ST15, ST273, ST2123, ST3449 and ST3520) were obtained. After ST identification, a phylogenetic tree was constructed (Fig. 2). The most common STs of CRKP resistant to CZA in our hospital are the following: ST11 (56·52%, 13/23), ST147 (13·04%, 3/23) and ST859 (8·70%, 2/23). Moreover, six ST11 strains, two ST859 strains, one ST273 strain, one ST3449 strain and one ST3520 strain carried the New Delhi metallo-β-lactamase-1 (*NDM-1*) gene. In addition, three ST147 strains and one ST15 strain carried the *NDM-5* gene.
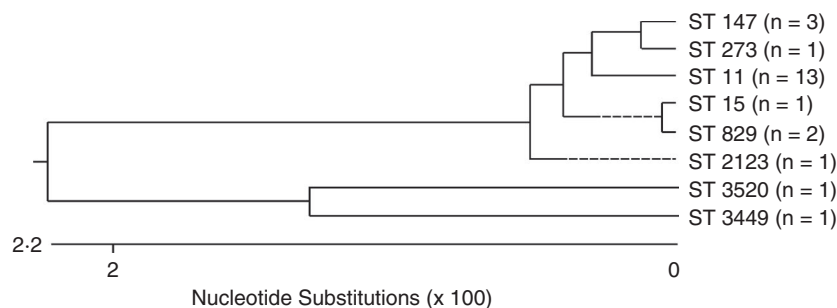
FIGURE 2 Multilocus sequence typing and phylogenetic analysis of carbapenem and ceftazidime-avibactam-resistant *Klebsiella pneumoniae*. Phylogenetic tree indicating the evolutionary relationships among the 23 strains of carbapenem and ceftazidime-avibactam-resistant *K. pneumoniae* strains isolated in this study. The frequency by which each sequence type was detected is indicated at each branch; ST, sequence type.
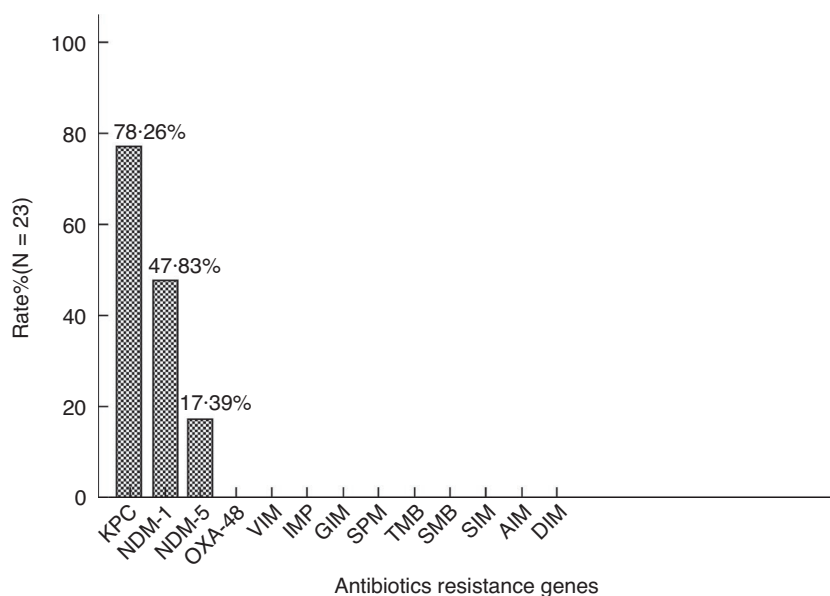

FIGURE 3 Antibiotic-resistant genes of carbapenem and ceftazidime-avibactam-resistant *Klebsiella pneumoniae*. Quantitative polymerase chain reaction was used to determine the frequency of 13 antibiotic-resistant genes in the strains of carbapenem and ceftazidime-avibactam-resistant *K. pneumoniae*. Bar plots indicate the frequency of each gene.

Prevalence of Ceftazidime Resistance Genes

The frequency of ceftazidime resistance genes in the 121 CRKP strains was investigated by real-time PCR. The *KPC* gene was found in 78·26% strains (18/23), the *NDM-1* gene was found in 47·83% strains (11/23) and the *NDM-5* gene was found in 17·39% (4/23) strains of resistant CRKP. On the other hand, *OXA-48*, *VIM*, *IMP*, *CIM*, *SPM*, *TMB*, *SMB*, *SIM*, *AIM* and *DIM* were not detected (Fig. 3).

DISCUSSION

In this study, we carried out a comprehensive examination of the characteristics of 121 CRKP strains and of the patients from which these were isolated. According to our analysis of clinical characteristics, patients with CRKP infection tended to be quite old, with an average of 72·54 years. Overall, elderly patients tend to be more susceptible to disease due to

low immunity and long hospital stays. This makes them more likely to contract an *Enterobacteriaceae* infection. Our study also found that 51·2% (62/121) of patients suffering from CRKP infection received treatment in the ICU. Many procedures in the ICU involve multiple organs, ventilator-assisted ventilation and invasive operations as well as the administration of a variety of antibacterial drugs, glucocorticoids and intravenous nutrient solutions. These large numbers and a wide variety of procedures performed in the ICU increases the patient's risk of contracting drug-resistant bacterial infections. Correlational research (Zheng *et al*. 2017; Liu *et al*. 2018; Li *et al*. 2019) has also found that immunosuppression, ICU admission, long hospital stays, antibiotic exposure, surgery and mechanical ventilation, as well as the application of central venous catheters, indwells and nasal catheters increase the risk of CRKP infection.

According to the China Antimicrobial Surveillance Network that monitors bacterial resistance (Simoons-Smit *et al*. 1986) and the European monitoring network of drug

resistance (Bush and Jacoby 2010), the increased rate of drug resistance and speed of CRKP development poses a serious threat to public health. Indeed, the drugs available for the treatment of CRKP are limited. Polymyxin and aminoglycoside are sparingly used in clinical applications owing to their ototoxicity and nephrotoxicity. On the other hand, the newly marketed CZA (Ehmann *et al.* 2012; Van Duin and Bonomo 2016) is a promising novel β-lactamase inhibitor, and it was been used in China by the end of 2019. Unlike classic β-lactamase inhibitors, avibactam does not contain a β-lactam core but still possesses the ability to covalently acylate its β-lactamase target. This occurs by the β-lactamase serine nucleophilic reaction that leads to ring opening and the formation of covalent compounds, resulting in complex enzyme inhibition without hydrolysis. As a result, β-lactamase activity is inhibited, and avibactam can recover from the reverse reaction and confer a long-acting inhibitory effect. In this study, 23 out of 121 strains (19·01%) of CRKP in our hospital were resistant to CZA, a higher proportion than the previously reported 3·7% (Zhang *et al.* 2020). This may be related to the fact that in the previous study, the CRKP samples were collected between 2014 and 2017, whereas ours were collected between 2017 and 2019. Carbapenemase-producing strains alter with age and further research needs to be conducted to determine whether metalloenzyme-producing strains change.

Molecular epidemiological data based on MLST showed that the STs of *K. pneumoniae* varied. STs were previously reported to be correlated with drug resistance; for example, *K. pneumoniae* ST258 is the ST most commonly resistant to carbapenems in the United States (Kitchel *et al.* 2009). In China, drug resistance to carbapenems is most common in ST11 (Wang *et al.* 2014). In this study, MLST results showed that CRKP resistance to CZA occurred in eight different STs, among which ST11 and ST147 were most abundant. If only one to three differentially expressed genotypes are found in the seven housekeeping genes, two STs are considered to be related. In this study, ST11, ST859, ST147 and ST273 had only one butler gene (*tonB*), which was closely related in the strains. Among the CZA-resistant CRKP, six strains of ST11, two strains of ST859, and one strain of ST273 possessed the *NDM-1* gene. Additionally, three strains of ST147 possessed the *NDM-5* gene. This suggests that ST11 may be more likely to obtain plasmids encoding the *NDM-1* gene, and ST147 may be more likely to obtain plasmids encoding the *NDM-5* gene. However, this hypothesis needs to be verified by further investigations with larger sample sizes. *VIM*, *IMP*, *CIM*, *SPM*, *TMB*, *SMB*, *SIM*, *AIM* and *DIM* were not detected, indicating that, at our hospital, the bla_{NDM-1} and bla_{NDM-5} genes were responsible for the development of CZA resistance in CRKP.

Ceftazidime-avibactam can inhibit A, C and some D enzymes *in vitro* but has no activity against B-type metallo-enzymes owing to the lack of serine residues at the active site. The metallo-β-lactamase of *K. pneumoniae* is encoded by many genes, including *NDM*, *IMP*, *VIM*, *SPM*, *GIM* and *SIM*. Genes that encode carbapenemase (Zou *et al.* 2015) are often located on mobile components, such as plasmids or transposons, which can be transferred between strains and species, making them highly transmissible. NDM was first detected in 2009 in a sample of *K. pneumoniae* from a Swiss patient who had travelled to India and had been hospitalized in New Delhi (Gu *et al.* 2018). Currently, 19 NDM subtypes, NDM-1 to NDM-19, have been identified (Livermore *et al.* 2011; Nordmann *et al.* 2012; Kazmierczak *et al.* 2016; Martin *et al.* 2017; Liu *et al.* 2019). Until 2013, Enterobacteriaceae with NDM-1-mediated carbapenem resistance were rarely reported in China (Hu *et al.* 2013), and most NDM-1-positive drug-resistant strains were found in and spread among travellers returning home. *Klebsiella pneumoniae* strains that produce the VIM enzyme are mainly found in Italy, Greece and other countries (Lauretti *et al.* 1999), whereas strains that produce the IMP enzyme are more common in China (Peirano *et al.* 2014). Bacteria carrying the *NDM* gene can develop resistance to a variety of antibiotics, including carbapenems. Therefore, these bacteria are known as 'super bacteria'. The high detection rate of *NDM* reported in this study suggests that *NDM* disseminates readily. In addition, owing to the correlation between *NDM* and resistance to multiple antibiotics, clinicians must strengthen the monitoring of these strains and continue to study the characteristics of clinical infection and transmission ability. By doing so, it may be possible to prevent and control infections by NDM-positive strains that occur during hospital stays.

Resistance to CZA is increasing via a number of different mechanisms (Barnes *et al.* 2017; Humphries and Hemarajata 2017; Gaibani *et al.* 2018; Galani *et al.* 2019; Hemarajata and Humphries 2019; Oueslati *et al.* 2019). For example, CZA resistance can be caused by mutations in the membrane porin OmpK35/36. Indeed, when KPC-23 isolates were analysed, they demonstrated increased CZA resistance due to increased ceftazidime hydrolysis and OmpK35 deficiency (Galani *et al.* 2019). Additionally, enhanced *K. pneumoniae* carbapenemase-3 (*KPC-3*) expression was reported in patients who had not been exposed to CZA but had previously been treated with meropenem and ceftazidime. The D179Y mutation of *KPC-3* resulted in the development of resistance against CZA. This mutation may be transferred to other microorganisms via plasmids (Gaibani *et al.* 2018). When the aspartic acid residue of KPC-2 was replaced by alanine, glutamine and asparagine, the resistance to CZA increased (Barnes *et al.* 2017). Additionally, the omega loop mutation (L169P) of KPC-2 has been linked to a case of CZA-resistant *K. pneumoniae* (Hemarajata and Humphries 2019). Moreover, a variant of KPC-2 with a Δ242-GT-243 mutation confers CZA resistance (Oueslati *et al.* 2019). Clinical isolates carrying

KPC-41 (defined by three amino acid insertions [Pro-Asn-Lys] between the amino acids 269 and 270 of KPC-3) also demonstrated CZA resistance. In addition, the carbapenemase activity of KPC-41 decreased dramatically (Mueller *et al.* 2019). In our study, the mechanism of CZA resistance was not determined for the eight strains identified. Further studies must be conducted on CZA-resistant CRKP to determine resistance and transmission mechanisms in strains that possess NDM-encoding genes.

In conclusion, the rate of CZA resistance in 121 CRKP strains obtained from the Fujian Provincial Hospital was 19·01% (23/121). ST11 was the main epidemic strain at our hospital. The presence of the carbapenemase genes bla_{KPC} and the metalloenzyme-encoding genes bla_{NDM-1} or bla_{NDM-5} correlated with an increased incidence of CZA resistance in CRKP.

CONFLICT OF INTEREST
No competing financial interest exists.

AUTHOR CONTRIBUTIONS
All authors contributed equally to this work. All authors read and approved the final article.

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REFERENCES
Barnes, M.D., Winkler, M.L., Taracila, M.A., Page, M.G., Desarbre, E., Kreiswirth, B.N., Shields, R.K., Nguyen, M.H. et al. (2017) *Klebsiella pneumoniae* carbapenemase-2 (KPC-2), substitutions at ambler position Asp179, and resistance to ceftazidime-avibactam: unique antibiotic-resistant phenotypes emerge from β-lactamase protein engineering. *MBio* 8, e00528-17.

Bush, K. and Jacoby, G.A. (2010) Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother* 54, 969–976.

Carmeli, Y., Armstrong, J., Laud, P.J., Newell, P., Stone, G., Wardman, A. and Gasink, L.B. (2016) Ceftazidime-avibactam or best available therapy in patients with ceftazidime-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* complicated urinary tract infections or complicated intra-abdominal infections (REPRISE): a randomized, pathogen-directed, phase 3 study. *The Lancet Infect Dis* 16, 661–673.

Diancourt, L., Passet, V., Verhoef, J., Grimont, P.A. and Brisse, S. (2005) Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 43, 4178–4182.

Ehmann, D.E., Jahić, H., Ross, P.L., Gu, R.F., Hu, J., Kern, G., Walkup, G.K. and Fisher, S.L. (2012) Avibactam is a covalent, reversible, non-β-lactam β-lactamase inhibitor. *Proc Natl Acad Sci* 109, 11663–11668.

Gaibani, P., Campoli, C., Lewis, R.E., Volpe, S.L., Scaltriti, E., Giannella, M., Pongolini, S., Berlingeri, A. et al. (2018) *In vivo* evolution of resistant subpopulations of KPC-producing *Klebsiella pneumoniae* during ceftazidime/avibactam treatment. *J Antimicrob Chemother* 73, 1525–1529.

Galani, I., Antoniadou, A., Karaiskos, I., Kontopoulou, K., Giamarellou, H. and Souli, M. (2019) Genomic characterization of a KPC-23-producing *Klebsiella pneumoniae* ST258 clinical isolate resistant to ceftazidime-avibactam. *Clin Microbiol Infect* 25, 763.e5–763.e8.

Gu, D., Dong, N., Zheng, Z., Lin, D., Huang, M., Wang, L., Chan, E.W.C., Shu, L. et al. (2018) A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. *Lancet Infect Dis* 18, 37–46.

Hemarajata, P. and Humphries, R.M. (2019) Ceftazidime/avibactam resistance associated with L169P mutation in the omega loop of KPC-2. *J Antimicrob Chemother* 74, 1241–1243.

Hu, L., Zhong, Q., Tu, J., Xu, Y., Qin, Z., Parsons, C., Zhang, B., Hu, X. et al. (2013) Emergence of bla_{NDM-1} among *Klebsiella pneumoniae* ST15 and novel ST1031 clinical isolates in China. *Diagn Microbiol Infect Dis* 75, 373–376.

Humphries, R.M. and Hemarajata, P. (2017) Resistance to ceftazidime-avibactam in *Klebsiella pneumoniae* due to porin mutations and the increased expression of KPC-3. *Antimicrob Agents Chemother* 61, https://doi.org/10.1128/AAC.00537-17.

Kazmierczak, K.M., Rabine, S., Hackel, M., McLaughlin, R.E., Biedenbach, D.J., Bouchillon, S.K., Sahm, D.F. and Bradford, P.A. (2016) Multiyear, multinational survey of the incidence and global distribution of metallo-β-lactamase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 60, 1067–1078.

Kitchel, B., Rasheed, J.K., Patel, J.B., Srinivasan, A., Navon-Venezia, S., Carmeli, Y., Brolund, A. and Giske, C.G. (2009) Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob Agents Chemother* 53, 3365–3370.

Lauretti, L., Riccio, M.L., Mazzariol, A., Cornaglia, G., Amicosante, G., Fontana, R. and Rossolini, G.M. (1999) Cloning and characterization of bla_{VIM}, a new integron-borne metallo-beta-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. *Antimicrob Agents Chemother* 43, 1584–1590.

Li, J., Li, Y., Song, N. and Chen, Y. (2019) Risk factors for carbapenem-resistant *Klebsiella pneumoniae* infection: a meta-analysis. *J Glob Antimicrob Resist* 24, 190–198.

Liu, P., Li, X., Luo, M., Xu, X., Su, K., Chen, S., Qing, Y., Li, Y. et al. (2018) Risk factors for carbapenem-resistant *Klebsiella pneumoniae* infection: a meta-analysis. *Microb Drug Resist* 24, 190–198.

Liu, Y., Zhang, H., Zhang, X., Jiang, N., Zhang, Z., Zhang, J., Zhu, B., Wang, G. et al. (2019) Characterization of an NDM-19-producing

Klebsiella pneumoniae strain harboring 2 resistance plasmids from China. *Diagn Microbiol Infect Dis* 93, 355–361.

Livermore, D.M., Mushtaq, S., Warner, M., Zhang, J., Maharjan, S., Doumith, M. and Woodford, N. (2011) Activities of NXL104 combinations with ceftazidime and aztreonam against carbapenemase-producing *Enterobacteriaceae*. *Antimicrob Agents Chemother* 55, 390–394.

Martin, J., Phan, H.T.T., Findlay, J., Stoesser, N., Pankhurst, L., Navickaite, I., De Maio, N., Eyre, D.W. et al. (2017) Covert dissemination of carbapenemase-producing *Klebsiella pneumoniae* (KPC) in a successfully controlled outbreak: long- and short-read whole-genome sequencing demonstrate multiple genetic modes of transmission. *J Antimicrob Chemother* 72, 3025–3034.

Mendes, R.E., Castanheira, M., Gasink, L., Stone, G.G., Nichols, W.W., Flamm, R.K. and Jones, R.N. (2015) *β*-Lactamase characterization of Gram-negative pathogens recovered from patients enrolled in the phase 2 trials for ceftazidime-avibactam: clinical efficacies analyzed against subsets of molecularly characterized isolates. *Antimicrob Agents Chemother* 60, 1328–1335.

Mueller, L., Masseron, A., Prod'Hom, G., Galperine, T., Greub, G., Poirel, L. and Nordmann, P. (2019) Phenotypic, biochemical and genetic analysis of KPC-41, a KPC-3 variant conferring resistance to ceftazidime-avibactam and exhibiting reduced carbapenemase activity. *Antimicrob Agents Chemother* 63, https://doi.org/10.1128/AAC.01111-19.

Nordmann, P., Boulanger, A.E. and Poirel, L. (2012) NDM-4 metallo-β-lactamase with increased carbapenemase activity from *Escherichia coli*. *Antimicrob Agents Chemother* 56, 2184–2186.

Oueslati, S., Iorga, B.I., Tlili, L., Exilie, C., Zavala, A., Dortet, L., Jousset, A.B., Bernabeu, S. et al. (2019) Unravelling ceftazidime/avibactam resistance of KPC-28, a KPC-2 variant lacking carbapenemase activity. *J Antimicrob Chemother* 74, 2239–2246.

Peirano, G., Lascols, C., Hackel, M., Hoban, D.J. and Pitout, J.D. (2014) Molecular epidemiology of *Enterobacteriaceae* that produce VIMs and IMPs from the SMART surveillance program. *Diagn Microbiol Infect Dis* 78, 277–281.

Simoons-Smit, A.M., Verweij-van, V.A.M. and MacLaren, D.M. (1986) The role of K antigens as virulence factors in *Klebsiella*. *J Med Microbiol* 21, 133–137.

Stone, G.G., Bradford, P.A., Newell, P. and Wardman, A. (2017) *In vitro* activity of ceftazidime-avibactam against isolates in a phase 3 open-label clinical trial for complicated intra-abdominal and urinary tract infections caused by ceftazidime-nonsusceptible Gram-negative pathogens. *Antimicrob Agents Chemother* 61, e01820-16.

Van Duin, D. and Bonomo, R.A. (2016) Ceftazidime/avibactam and Ceftolozane/tazobactam: second-generation β-lactam/β-lactamase inhibitor combinations. *Clin Infect Dis* 63, 234–241.

Wang, X., Li, H., Zhao, C., Chen, H., Liu, J., Wang, Z., Wang, Q., Zhang, Y. et al. (2014) Novel NDM-9 metallo-β-lactamase identified from a ST107 *Klebsiella pneumoniae* strain isolated in China. *Int J Antimicrob Agents* 44, 90–91.

Zhang, P., Shi, Q., Hu, H., Hong, B., Wu, X., Du, X., Akova, M. and Yu, Y. (2020) Emergence of ceftazidime/avibactam resistance in carbapenem-resistant *Klebsiella pneumoniae* in China. *Clin Microbiol Infect* 26, 124.e1–124.e4.

Zheng, B., Dai, Y., Liu, Y., Shi, W., Dai, E., Han, Y., Zheng, D., Yu, Y. et al. (2017) Molecular epidemiology and risk factors of carbapenem-resistant *Klebsiella pneumoniae* infections in Eastern China. *Front Microbiol* 8, 1061.

Zou, D., Huang, Y., Zhao, X., Liu, W., Dong, D., Li, H., Wang, X., Huang, S. et al. (2015) A novel New Delhi metallo-β-lactamase variant, NDM-14, isolated in a Chinese Hospital possesses increased enzymatic activity against carbapenems. *Antimicrob Agents Chemother* 59, 2450–2453.