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CASE REPORT

A Novel Variant of KPC-179 Conferring Ceftazidime-Avibactam Resistance in a Carbapenem-Resistant *Klebsiella pneumoniae* Isolate

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Objective: Ceftazidime-avibactam (CZA) is a novel β-lactam/β-lactamase inhibitor with activity against carbapenem-resistant *Klebsiella pneumoniae* (CRKP) that produce *Klebsiella pneumoniae* carbapenemase (KPC). In this study, we report the first cases of CZA resistance to develop during treatment of CRKP infections and identify the resistance mechanism.

Methods: APB/EDTA and NG-Test CARBA5 were used to detect the production of carbapenemase, whole-genome sequencing (WGS) and conjugation experiment were used to identify potential resistance mechanisms of CZA-susceptible (HX1032) and -resistant (HX1192) *K. pneumoniae* isolates.

Results: HX1192 *K. pneumoniae* was not recognized by APB/EDTA and NG-Test CARBA5 phenotypic assays, WGS revealed it carrying a novel KPC variant, KPC-179, molecular analysis highlighted a G394A mutation, and an ATC insertion at 543 in the bla_{KPC-2} gene, resulting in an A133T substitution and insertion of the amino acid S at Ambler position 183 in the protein sequence. Remarkably, this mutation restored susceptibility of imipenem (MIC = 0.25 mg/L).

Conclusion: Our study highlights the importance of monitoring susceptibility during CZA treatment and accurately detecting KPC variants.

Keywords: CZA resistance, carbapenem-resistant, *Klebsiella pneumoniae*, KPC variant, phenotypic test

Introduction

Carbapenem-resistant *K. pneumoniae* (CRKP) strains are among the most serious antimicrobial resistant strains worldwide, leading to high morbidity and mortality.[1] The main carbapenemase in CRKP is *Klebsiella pneumoniae* carbapenemase (KPC), which hydrolyzes almost all cephalosporins and carbapenems, resulting in a lack of effective treatment options.[2] As a result, tigecycline and polymyxin B have become the final resorts for defense against multidrug-resistant gram-negative bacteria.[3] However, side effects, such as toxicity and induced resistance, limit their clinical application.[4]

Ceftazidime–avibactam (CZA) is a novel combination of β-lactam and β-lactamase inhibitors, and offers a valuable alternative strategy against KPC-producing *K. pneumoniae* infections.[5] Avibactam protects ceftazidime from hydrolysis by KPCs, OXA-48, AmpC, and extended-spectrum β-lactamases (ESBLs) but not metallo-β-lactamases.[6] Reports of resistance to CZA have increased in recent years since clinical approval.[7,8] The main resistance mechanism of CZA is mutation, which is derived from the bla_{KPC-2} or bla_{KPC-3} genes, causing abnormal amino acids in the protein sequences, and loss of outer membrane proteins, mutations in other β-lactamases genes and overexpression of KPC enzyme and efflux pumps could also lead to CZA resistance.[7–9] Moreover, the number of newly identified KPC variants increases within the last 2 years, more than 190 bla_{KPC} subtypes have been reported in the world according to the NCBI database until Jan. 2024.

Here, we describe the characterization of $bla_{KPC-179}$, a novel bla_{KPC} variant that confers resistance to CZA while restoring susceptibility to certain carbapenems.

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Patients and Methods

Patient

Clinical data were extracted from the hospital information system (HIS) were extracted, including age, sex, specimen origin, antibiotic exposure, duration of hospitalization, in-hospital stay, and disease prognosis.

Phenotypic Detection and Antimicrobial Susceptibility Testing

Two CRKP strains, HX1032 and HX1192, were isolated from the sputum specimens of a patient hospitalized at West China Hospital in Sichuan, China. The isolates were confirmed as *K. pneumoniae* using a Bruker Biotyper MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). Minimum inhibitory concentration (MIC) results were determined by VITEK-2 automatic microbial analyzer (BioMerieux, Marcy-l'Étoile, France) and broth microdilution, *K. pneumoniae* ATCC 700603, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC27853 were used as quality control strains, and the results were interpreted by CLSI 2022 for all agents, except for tigecycline and eravacycline, which were interpreted according to the guidelines of the Food and Drug Administration, and aztreonam-avibactam was interpreted by European Committee on Antimicrobial Susceptibility Testing. Carbapenemase production was phenotypically detected using APB/EDTA[10] (carbapenemase inhibitor APB [3-aminophenylboronic acid] and EDTA enhancement method) and NG-Test CARBA 5.[11]

Conjugation Experiments

A conjugation experiment[12] was conducted with rifampicin-resistant *E. coli* EC600 as a recipient strain to explore the transferability of CZA resistance. The transconjugants were screened on Luria–Bertani agar plates containing rifampicin (600 mg/L) and CZA (8 mg/L). The plates were incubated at 37°C for 16–18 h. PCR was performed to confirm the presence of bla_{KPC}.

Whole-Genome Sequencing (WGS) and Analysis

Genomic DNAs of the CRKP strains were extracted using a QIAamp1 DNA Mini Kit (QIAGEN, Hilden, Germany) and subsequently sequenced using the Illumina HiSeq X10 platform (Illumina Inc., San Diego, CA). The sickle tool (GitHub) and SPAdes 3.8 were utilized for sequence trimming and de novo genome assembly. Sequence typing (ST) was performed using Institut Pasteur (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Antimicrobial resistance genes were identified using the ABRicate program (https://github.com/tseemann/abricate) to query the ResFinder database (http://genomicepidemiology.org/).[13]

Results

The patient was a 40-year-old male with bacterial pneumonia (Carbapenem-resistant *Klebsiella pneumoniae*), IVB stage NK/T-cell lymphoma, septic shock, sepsis, and hemophagocytic syndrome. He was in poor condition and experienced recurrent fever. Piperacillin-tazobactam (4.5 g every 8 h) was administered on Jun. 14, Meropenem (1000 mg every 8 h) and sulfamethoxazole/trimethoprim (0.48 g once a day) were substituted with piperacillin-tazobactam because of fever and immunosuppression after 8 days. On day 16, the antibiotic regimen was changed to imipenem-cilastatin (500 mg every 6 h). On Sep. 17, the first CRKP isolate (HX1032, bla_{KPC-2} positive) was recovered from the sputum sample. Due to the patient's symptoms and drug susceptibility results, imipenem was replaced with tigecycline (100 mg every 12 h), and CZA (2.5 g every 8 h) was added due to rising body temperature after six days. The second CRKP strain (HX1192, $bla_{KPC-179}$ positive) was isolated from the sputum on Nov. 15. Antibiotics were adjusted multiple times, considering side effects, recurrent clinical symptoms, and the patient's economic condition. However, the patient eventually died. Microbiological details, timelines, and antibiotic therapies used are summarized in Figure 1.

HX1032 and HX1192 were bla_{KPC}-positive isolates highly resistant to β-lactams, aminoglycosides, quinolones, and eravacycline. However, HX1032 was sensitive to colistin, tigecycline, CZA, and aztreonam-avibactam, and phenotypic detection revealed that HX1032 produces serine-carbapenemase (KPC). In contrast, while HX1192 was resistant to CZA, the hydrolytic ability of imipenem was restored and the MIC of meropenem decreased. APB/EDTA and NG-Test CARBA 5 were unable to distinguish the carbapenemase of HX1192 carbapenemases. The plasmid harboring $bla_{KPC-179}$ from strain HX1192 was successfully transferred into the *E. coli* EC600 recipient strain, rendering the

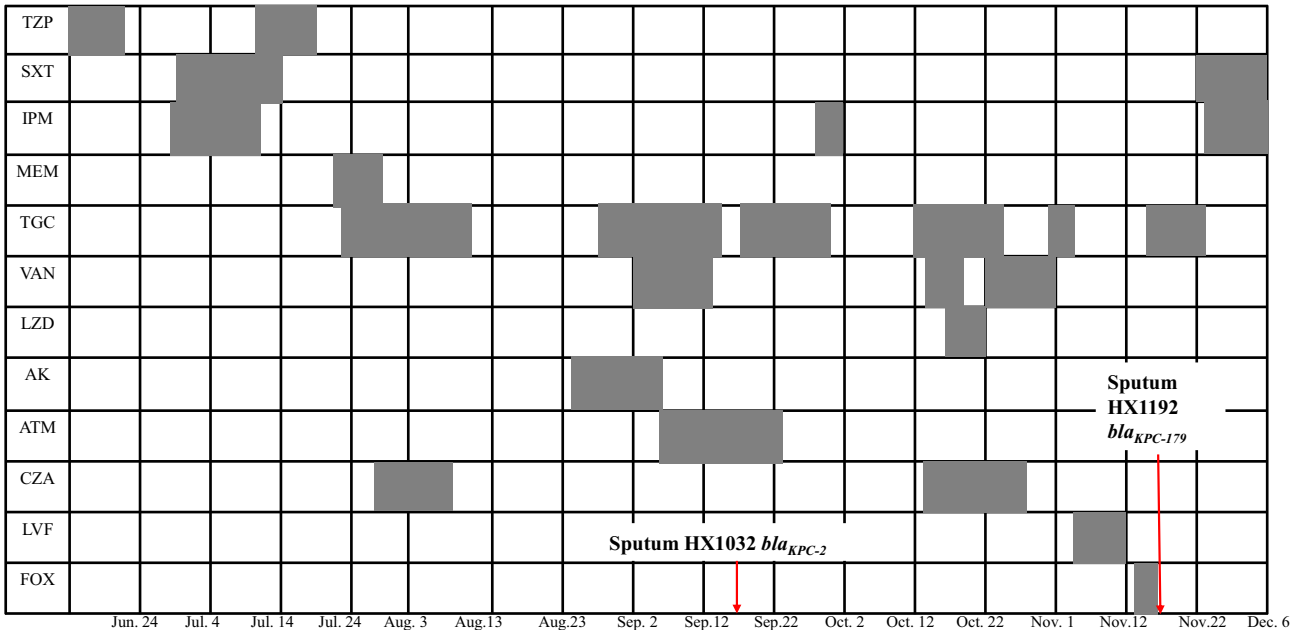

Figure 1 Antimicrobial treatments of the patient and isolation of *K. pneumoniae* strains.
Abbreviations: TZP, piperacillin-tazobactam; SXT, sulfamethoxazole/trimethoprim; IMP, imipenem-cilastatin; MEM, meropenem; ATM, aztreonam; TGC, tigecycline; VAN, vancomycin; LZD, linezolid; AK, amikacin; CZA, ceftazidime-avibactam; LVF, levofloxacin; FOX, cefoxitin.

transconjugants resistant to CZA, quinolones, aminoglycosides, and cephalosporins (Table 1). Despite HX1192 CRKP was resistant to ertapenem, we retested HX1192 using APB/EDTA with ertapenem. However, the enzyme type remained undetectable (Figure 2).

Table 1 Minimal Inhibitory Concentrations (MICs) of K. Pneumoniae Strains HX1032, HX1192, and the $bla_{KPC-179}$-Positive E. Coli Transconjugant of HX1192

Antimicrobial Agents	MIC (mg/L)			
	***K. Pneumoniae* HX1032 (*bla*$_{KPC-2}$)**	***K. Pneumoniae* HX1192 (*bla*$_{KPC-179}$)**	**Transconjugant *E. Coli* HX1192-EC600 (*bla*$_{KPC-179}$)**	***E. Coli* EC600**
Piperacillin-tazobactam	≥128	≥128	16	≤4
Ampicillin-sulbactam	≥32	≥32	≥32	4
Cefpodoxime	≥8	≥8	≥8	1
Ceftriaxone	≥64	≥64	≥64	≤1
Cefepime	≥64	≥64	4	≤1
Ceftazidime	≥64	≥64	≥64	≤1
Aztreonam	≥64	≥64	≥64	≤1
Meropenem	≥16	2	≤0.25	≤0.25
Ertapenem	≥8	≥8	≤0.5	≤0.5
Imipenem	≥16	0.25	≤0.25	≤0.25
Amikacin	≥64	≥64	≥64	≤2
Gentamicin	≥16	≥16	≥16	≤1
Ciprofloxacin	≥4	≥4	2	≤0.25
Levofloxacin	≥8	≥8	4	0.5
Colistin	0.5	1	0.5	1
Tigecycline	1	0.5	≤0.5	≤0.5
Ceftazidime-avibactam (AVI 4)	4	≥256	32	0.125
Eravacycline	2	1	0.5	0.25
Aztreonam-avibactam	1	1	0.5	0.125

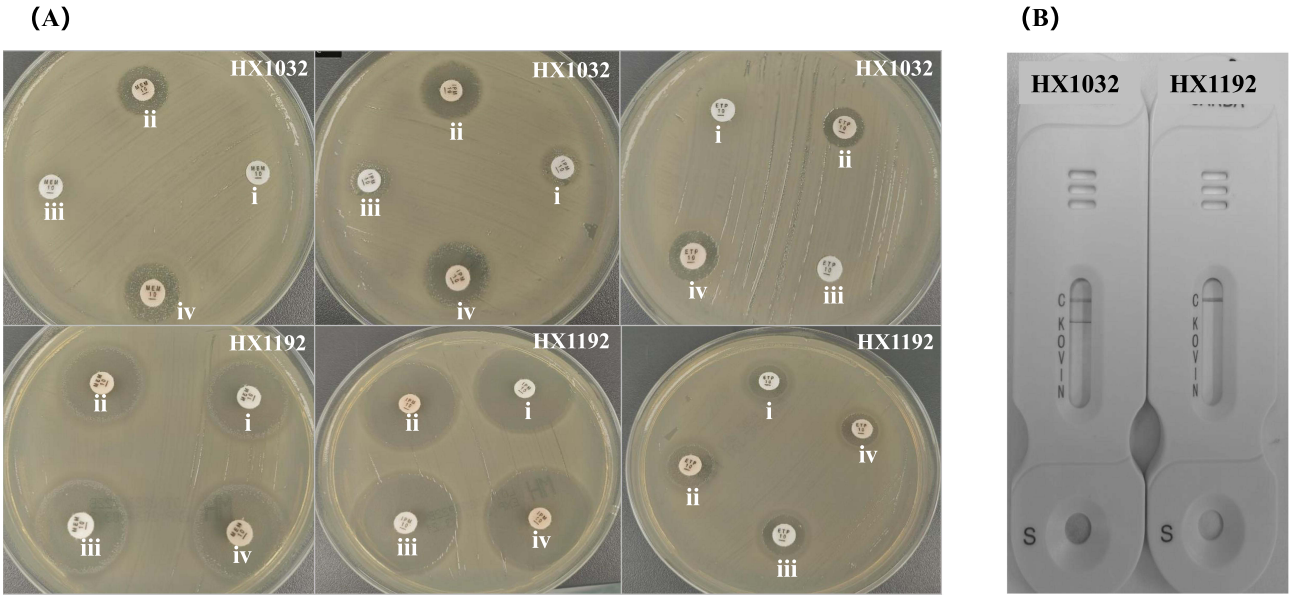

Figure 2 Carbapenemase enzyme detection with APB/EDTA (**A**) and NG-Test CARBA 5 (**B**).
Notes: (**A**); i, carbapenem; ii, carbapenem + APB; iii, carbapenem + EDTA; iv, carbapenem + APB+ EDTA. HX1032 strain produced serine-carbapenemase (KPC) and HX1192 strain could not be distinguished by APB/EDTA and NG-Test CARBA 5.

Based on the results of WGS analysis, *K. pneumoniae* HX1032 belonged to clonal lineage ST11 and carried the bla_{KPC-2} gene. Isolate HX1192 also belonged to ST11 and harbored $bla_{KPC-179}$ (a variant of bla_{KPC-2}). Other resistance genes explaining the resistance phenotype of the two CRKP isolates are shown in Table 2.

Discussion

As one of the few effective treatment alternatives against KPC-producing *K. pneumoniae* strains, CZA has been widely used clinically since its approval.[14] However, acquired resistance has been increasingly reported in multiple independent occurrences. The primary resistance mechanisms include (1) the co-production of serine-carbapenemase and metallo-*β*-lactamases,[15] (2) overexpression of bla_{KPC}, researches showed increased expression of $bla_{\text{KPC-2}}$ and $bla_{\text{KPC-3}}$ could contribute to resistance to CZA,[16–18] (3) membrane impermeability of porin mutations, including mutation of the ompK35/36 gene resulting in a significant increase (4→32 mg/L) in the MIC value of CZA in *K. pneumonia*e,[18] and OprD loss with elevated AmpC could cause resistance to CZA in *P. aeruginos*a,[19] (4) overexpression of efflux pumps, increased activity of the MexAB–OprM efflux system, resulting from overexpression of *mexA* and overproduction of AmpC may be the mechanism underlying the resistance of CZA,[8] (5) mutations in other *β*-lactamases genes, such as $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{OXA}, AmpC enzyme, and the Leu169Pro amino acid substitution in PBP3 mutations were related to CZA resistance,[8] and (6) mutations derived from the bla_{KPC-2} or bla_{KPC-3} gene, resulting in amino acid substitutions at positions 164, 167, 169, and 179 within the

Table 2 Molecular Characteristics of CRKP Isolates from the Present Study

Isolate	MLST	Carbapenemases	Other *β*-lactamase genes	Other Resistance Genes	Plasmid IncC
HX1032	ST 11	KPC-2	$bla_{SHV-187}$, bla_{SHV-12}, $bla_{SHV-158}$, bla_{LAP-2}, bla_{TEM-1}, $bla_{CTX-M-65}$	*aadA2, sul1, catA2, dfrA14, fosA6, qnrS1, sul2, rmtB1, tet(A)*	pENTAS02_1, IncHI1B_1_pNDM-MAR, IncFIB(K)_1_Kpn3, IncR_1, IncFII(pCRY)_1_pCRY, ColRNAI_1, IncFII(pHN7A8)_1_pHN7A8
HX1192	ST 11	KPC-179 (A133T + 183S insertion)	$bla_{SHV-187}$, bla_{SHV-12}, $bla^{SHV-158}$, bla_{LAP-2}, bla_{TEM-1}, $bla_{CTX-M-65}$	*catA2, fosA6, qnrS1, sul2, rmtB1, tet(A), aadA2, sul1*	pENTAS02_1, IncHI1B_1_pNDM-MAR, IncFIB(K)_1_Kpn3, IncR_1, IncFII(pCRY)_1_pCRY, ColRNAI_1, IncFII(pHN7A8)_1_pHN7A8

Ω-loop of class A *β*-lactamases, which are the most frequent mutations, followed by amino acid insertions and deletions.[12,20,21]

In the present study, we identified a CRKP (HX1192) isolate harboring $bla_{KPC-179}$ (NCBI number, OR115556.1), a novel KPC-2 variant of clinical origin associated with reduced susceptibility to CZA (MIC ≥ 256 mg/L) during the treatment of CRKP infections, and its MICs of imipenem and meropenem decreased to 0.25 mg/L and 2 mg/L respectively, which was consistent with previous reports that strains restored susceptibility to imipenem/meropenem or low-level resistance to meropenem,[12,13] the reason was the mutations within the bla_{KPC} Ω-loop (positions 165–179) enhance ceftazidime affinity and restrict avibactam binding.[12] However, HX1192 carried an A133T substitution and an insertion of S at Ambler position 183 in the protein sequence. Whether these mutations could cause a structural change in the KPC protein, similar to previously observed mutations, resulting in CZA resistance and the recovery of carbapenem susceptibility warrants further investigation.

In our case, $bla_{KPC-179}$-positive CRKP was isolated after using CZA for more than two weeks, and we previously identified a CRKP carrying bla_{KPC-78} after 14 days of CZA treatment in a 58-year-old male,[13] Shi Q[12] reported that after 16 days of CZA use, a bla_{KPC-33}-harboring *Klebsiella pneumoniae* was detected in sputum. Three patients carrying bla_{KPC-7}, $bla_{KPC-179}$ and bla_{KPC-33} CRKP isolates were treated with CZA for more than seven days. We speculate that the duration of CZA usage may be associated with the selective pressure of bacteria, thus causing CZA resistance, and more evidences were needed. Therefore, clinicians should be vigilant for CZA resistance when CZA is used to treat infections caused by bla_{KPC-2}-positive isolates.

The Infectious Diseases Society of America (IDSA) recommended CZA, eravacycline, colistin, tigecycline, CZA, and aztreonam (exert the action of aztreonam-avibactam) for the treatment of carbapenem-resistant *Enterobacterales* -associated infections.[22] However, colistin and tigecycline have limited clinical use because of their side effects. Newer drugs, such as aztreonam-avibactam, have emerged as alternatives, specifically targeting CZA-resistant strains. HX1032 and HX1192 are resistant to *β*-lactams, aminoglycosides, and quinolones. In this case, colistin, tigecycline, CZA, aztreonam-avibactam, and eravacycline may have been therapeutic agents. Aztreonam-avibactam is considered useful for the treatment of infections caused by gram-negative organisms, particularly those that produce MBLs and serine carbapenemases.[23] In this study, aztreonam-avibactam demonstrated potent antibacterial activity against KPC-mutant CRKP strains in vitro.

KPC serine carbapenemases, MBLs, and the coproduction of MBLs and KPC enzymes can be distinguished using APB/EDTA in combination with imipenem or meropenem.[10] HX1032 carries serine carbapenemase via APB/EDTA with imipenem, meropenem, and ertapenem. However, the presence of serine carbapenemase in HX1192 was not confirmed by APB/EDTA with imipenem, meropenem, and ertapenem, which could be attributed to bla_{KPC-2} mutations resulting in the loss of carbapenemase activity. Our results suggested that strains harboring KPC variants may escape through surveillance procedures, thus facilitating their spread in hospital settings, making it particularly important to select suitable negativity of phenotypical carbapenemase detection methods. Immunochromatographic assay NG-Test CARBA 5 detected common carbapenemase genes (KPC, NDM, VIM, IMP, and OXA-48) and some KPC mutations, such as bla_{KPC-79} (262V_268N dup) and bla_{KPC-35} (L169P), but not bla_{KPC-33} (D179Y), bla_{KPC-71}(181S_182P insertion), bla_{KPC-76} (D179Y+262V_268N dup), or $bla_{KPC-179}$ (A133T+183S insertion).[24] These findings suggested that position 179 or the surrounding amino acids in KPC are crucial for accurate enzyme immunodetection. However, other resistance mechanisms such as porin loss or mutations in other *β*-lactamases genes cannot be detected by immunochromatographic assays, and alternative methods such as Chromatic Super CAZ/AVI® medium[25] could be considered, using molecular biological approaches if necessary.

This study has some limitations. First, other resistance genes in HX1032 and HX1192 *K. pneumoniae* strains were not analyzed. Second, our study did not use structural and biochemical assays to characterize the functional consequences of $bla_{KPC-179}$.

Conclusions

In summary, we identified a novel KPC-2 variant, KPC-179, that emerged during CZA treatment. Clinicians must tailor the treatment plans for critically ill patients, closely monitor the susceptibility of KPC-producing strains to CZA, and promptly detect KPC variants at an early stage of therapy. This approach will help prevent treatment failure and the rapid dissemination of CZA-resistant strains.

Data Sharing Statement

The datasets presented in this study are available in the NCBI Sequence Read Archive (SRA) under accession number PRJNA1086695.

Ethical Statement and Informed Consent

All experimental protocols were approved by the Ethics Committee of the West China Hospital of Sichuan University, and the number was 2021623. Institutional approval wasn't required to publish the case details. The patient provided written informed consent for publication of this case report.

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Disclosure

The authors declare that they have no conflict of interest.

References

1. Tsioutis C, Eichel VM, Mutters NT. Transmission of Klebsiella pneumoniae carbapenemase (KPC)-producing Klebsiella pneumoniae: the role of infection control. *J Antimicrob Chemother*. 2021;76(Suppl 1):i4–i11.
2. Liao W, Liu Y, Zhang W. Virulence evolution, molecular mechanisms of resistance and prevalence of ST11 carbapenem-resistant Klebsiella pneumoniae in China: a review over the last 10 years. *J Glob Antimicrob Resist*. 2020;23:174–180. doi:10.1016/j.jgar.2020.09.004
3. Jean SS, Harnod D, Hsueh PR. Global Threat of Carbapenem-Resistant Gram-Negative Bacteria. *Front Cell Infect Microbiol*. 2022;12:823684. doi:10.3389/fcimb.2022.823684
4. Yamamoto M, Pop-Vicas AE. Treatment for infections with carbapenem-resistant Enterobacteriaceae: what options do we still have? *Crit Care*. 2014;18(3):229. doi:10.1186/cc13949
5. Boattini MBG, Bianco G, Charrier L, et al. Rapid diagnostics and ceftazidime/avibactam for KPC-producing Klebsiella pneumoniae bloodstream infections: impact on mortality and role of combination therapy. *Eur J Clin Microbiol Infect Dis*. 2023;42(4):431–439. doi:10.1007/s10096-023-04577-x
6. Shirley M. Ceftazidime-Avibactam: a Review in the Treatment of Serious Gram-Negative Bacterial Infections. *Drugs*. 2018;78(6):675–692. doi:10.1007/s40265-018-0902-x
7. Wang Y, Wang J, Wang R, Cai Y. Resistance to ceftazidime-avibactam and underlying mechanisms. *J Glob Antimicrob Resist*. 2020;22:18–27. doi:10.1016/j.jgar.2019.12.009
8. Xiong L, Wang X, Wang Y, et al. Molecular mechanisms underlying bacterial resistance to ceftazidime/avibactam. *WIREs Mech Dis*. 2022;14(6):e1571. doi:10.1002/wsbm.1571
9. Shields RK, Nguyen MH, Press EG, Chen L, Kreiswirth BN, Clancy CJ. Emergence of Ceftazidime-Avibactam Resistance and Restoration of Carbapenem Susceptibility in Klebsiella pneumoniae Carbapenemase-Producing K pneumoniae: a Case Report and Review of Literature. *Open Forum Infect Dis*. 2017;4(3):ofx101. doi:10.1093/ofid/ofx101
10. Liao Q, Yuan Y, Li Q, et al. Comparing three different phenotypic methods for accurate detection of carbapenemase-producing Enterobacterales. *J Infect Chemother*. 2021;27(6):794–799. doi:10.1016/j.jiac.2021.01.003
11. Huang Y-WK Y-T, Lee N-Y, Tien N, et al. SMART study group: evaluating NG-Test CARBA 5 Multiplex Immunochromatographic and Cepheid Xpert CARBA-R Assays among Carbapenem-Resistant Enterobacterales Isolates Associated with Bloodstream Infection. *Microbiol Spectr*. 2022;10(1):e0172821. doi:10.1128/spectrum.01728-21
12. Qingyu Shi DY, Han R, Guo Y, et al. Emergence and Recovery of Ceftazidime-avibactam Resistance in blaKPC-33-Harboring Klebsiella pneumoniae Sequence Type 11 Isolates in China. *Clin Infect Dis*. 2020;71(Suppl 4):S436–S439. doi:10.1093/cid/ciaa1521
13. Quanfeng Liao JD, Feng Y, Zhang W, Siying W, Liu Y, Che H. Yi Xie: emergence of ceftazidime-avibactam resistance due to a novel blaKPC-2 mutation during treatment of carbapenem-resistant Klebsiella pneumoniae infections. *J Infect Public Health*. 2022;15(5):545–549. doi:10.1016/j.jiph.2022.04.002
14. Tumbarello M, Raffaelli F, Giannella M, et al. Ceftazidime-Avibactam Use for Klebsiella pneumoniae Carbapenemase-Producing K. pneumoniae Infections: a Retrospective Observational Multicenter Study. *Clin Infect Dis*. 2021;73(9):1664–1676. doi:10.1093/cid/ciab176
15. Rong F, Liu Z, Yang P, et al. Epidemiological and Molecular Characteristics of bla (NDM-1) and bla (KPC-2) Co-Occurrence Carbapenem-Resistant Klebsiella pneumoniae. *Infect Drug Resist*. 2023;16:2247–2258. doi:10.2147/IDR.S400138
16. Zj LX, Yang C, Li J, et al. Increased Expression and Amplification of blaKPC-2 Contributes to Resistance to Ceftazidime/Avibactam in a Sequence Type 11 Carbapenem-Resistant Klebsiella pneumoniae Strain. *Microbiol Spectr*. 2022;10(4):e0095522. doi:10.1128/spectrum.00955-22
17. Sq ZP, Hu H, Hong B, et al. Emergence of ceftazidime/avibactam resistance in carbapenem-resistant Klebsiella pneumoniae in China. *Clin Microbiol Infect*. 2020;26(1):124.e1–124.e4. doi:10.1016/j.cmi.2019.08.020
18. Hp HRM, Hemarajata P. Resistance to Ceftazidime-Avibactam in Klebsiella pneumoniae Due to Porin Mutations and the Increased Expression of KPC-3. *Antimicrob Agents Chemother*. 2017;61(6):e00537–17. doi:10.1128/AAC.00537-17

19. Hk ZR, Joshi C, Aitken E, Oggioni MR, Gould IM, Gould IM. Phylogenetic analysis of resistance to ceftazidime/avibactam, ceftolozane/tazobactam and carbapenems in piperacillin/tazobactam-resistant Pseudomonas aeruginosa from cystic fibrosis patients. *Int J Antimicrob Agents*. 2019;53(6):774–780. doi:10.1016/j.ijantimicag.2019.02.022
20. Di Bella S, Giacobbe DR, Maraolo AE, et al. Resistance to ceftazidime/avibactam in infections and colonisations by KPC-producing Enterobacterales: a systematic review of observational clinical studies. *J Glob Antimicrob Resist*. 2021;25:268–281. doi:10.1016/j.jgar.2021.04.001
21. Hobson CA, Pierrat G, Tenaillon O, et al. Klebsiella pneumoniae Carbapenemase Variants Resistant to Ceftazidime-Avibactam: an Evolutionary Overview. *Antimicrob Agents Chemother*. 2022;66(9):e0044722. doi:10.1128/aac.00447-22
22. Tamma PD, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ, Clancy CJ. Infectious Diseases Society of America 2022 Guidance on the Treatment of Extended-Spectrum β-lactamase Producing Enterobacterales (ESBL-E), Carbapenem-Resistant Enterobacterales (CRE), and Pseudomonas aeruginosa with Difficult-to-Treat Resistance (DTR-P. aeruginosa). *Clin Infect Dis*. 2022;75(2):187–212. doi:10.1093/cid/ciac268
23. Sader HS, Ryan Arends SJ, Castanheira M, Mendes RE, Mendes RE. Aztreonam/avibactam activity against clinical isolates of Enterobacterales collected in Europe, Asia and Latin America in 2019. *J Antimicrob Chemother*. 2021;76(3):659–666. doi:10.1093/jac/dkaa504
24. Li Ding QS, Han R, Yin D, et al. Comparison of Four Carbapenemase Detection Methods for blaKPC-2 Variants. *Microbiol Spectr*. 2021;9(3):e00954–21. doi:10.1128/Spectrum.00954-21
25. Bianco GBM, Comini S, Leone A, et al. Implementation of Chromatic Super CAZ/AVI® medium for active surveillance of ceftazidime-avibactam resistance: preventing the loop from becoming a spiral. *Eur J Clin Microbiol Infect Dis*. 2022;41(9):1165–1171. doi:10.1007/s10096-022-04480-x