Ceftazidime/Avibactam Resistance in Carbapenemase-Producing *Klebsiella pneumoniae*

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We identified a novel ceftazidime/avibactam resistance mechanism in sequence type 11 *Klebsiella pneumoniae* carbapenemase 2–producing *K. pneumoniae*. Plasmid recombination and chromosomal integration formed a novel virulence plasmid and provided an additional promoter for bla_{SHV-12}, leading to bla_{SHV-12} overexpression and ceftazidime/avibactam resistance. Genetic rearrangement contributed to convergence of hypervirulence and ceftazidime/avibactam resistance.

Emergence and global dissemination of carbapenem-resistant *Klebsiella pneumoniae* pose therapeutic challenges to public health (*1*). The most crucial cause of carbapenem resistance in *K. pneumoniae* is carbapenemase production; thus, the novel β-lactamase inhibitor ceftazidime/avibactam (CAZ/AVI) provides an antimicrobial strategy (*1*–*3*). However, its increasing use raises resistance concerns. According to the China Antimicrobial Surveillance Network (http://www.chinets.com/Data/AntibioticdrugFast), 9.9% of *K. pneumoniae* carbapenemase (KPC) 2–producing *K. pneumoniae* (KPC-KP) displayed CAZ/AVI resistance (*4*). β-lactamase amino acid substitutions are the dominant mechanisms that lead to CAZ/AVI resistance (*5*). Mutations in class A β-lactamases, especially KPCs, have been reported (*5*). Substitutions in KPCs could improve ceftazidime affinity or reduce avibactam inhibition (*5*). We report a novel CAZ/AVI resistance mechanism in epidemic sequence type (ST) 11 KPC-KP. All study procedures involving human participants and animals were in accordance with the ethics standards of the Institutional Review Board Ethics Committee of Shanxi Provincial People's Hospital; this type of retrospective study did not require formal consent.

In 2021, a 62-year-old man was transferred from another hospital to a teaching hospital in Shanxi Province, China. Before transfer, a blood culture indicated CAZ/AVI–susceptible carbapenem-resistant *K. pneumoniae*. The patient received 1 week of CAZ/AVI therapy before transfer and another week of CAZ/AVI therapy after admission. In addition to the bloodstream infection, severe pneumoniae, multiple duodenal ulcers, and gastrointestinal hemorrhage de-

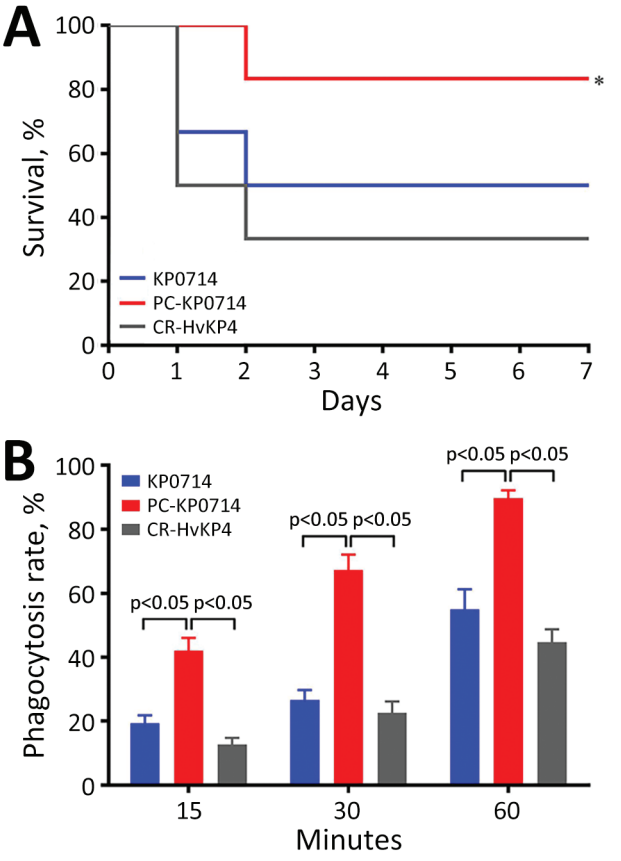

Figure 1. Linear alignment of plasmid pVir-KP0714 and virulence potential determination of *Klebsiella. pneumoniae* isolate KP0714 in study of ceftazidime/avibactam resistance in carbapenemase-producing *K. pneumoniae*. A) Virulence potential determination of KP0714 and pVir-KP0714–curing mutant (PC-KP0714) in a mouse infection model. Sequence type 11 carbapenem-resistant hypervirulent *K. pneumoniae* strain CR-HvKP4 was used as a hypervirulence control. Bacterial suspensions in the logarithmic growth phase were diluted in sterile phosphate-buffered saline to 10^7 CFU/mL. Six female BALB/c mice were used as a sample population for each isolate. BALB/c mice were infected intraperitoneally with 0.1 mL of the diluted bacterial suspension. Clinical signs and mortality rates were noted for 7 days. *p<0.05 when compared with PC-KP0714. B) Human neutrophil assays of KP0714. Error bars indicate SDs. p values were computed by 1-way analysis of variance with Bonferroni correction.

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veloped. Two weeks after CAZ/AVI withdrawal, we isolated KP0714, which was resistant to all β-lactams tested but susceptible to tigecycline and polymyxin B (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/29/11/23-0830-App1.pdf). We generated the KP0714 complete genome by using the combination of Illumina and PacBio RS sequencing (Appendix Table 2), and it belonged to ST11. The resistance plasmid pKPC-KP0714 carries bla_{KPC-2} and several other resistance genes, including bla_{TEM-1}, *rmtB*, and *fosA3* (Appendix Table 2), and KPC-2 S130A substitution was constructed in situ. KP0714 and the KPC-2 S130A mutant displayed the same MICs for CAZ/AVI, suggesting that bla_{KPC-2} was not involved in CAZ/AVI resistance (Appendix Table 1).

KP0714 possessed a novel IncFIB(K)-type virulence plasmid pVir-KP0714, encoding siderophore aerobactin (*iucABCDiutA*) and capsular polysaccharide regulator RmpA2. pVir-KP0714 was 99.97% identical to reference plasmid pOXA1_020030 (GenBank accession no. CP028791) from *K. pneumoniae* strain WCHKP020030 at 74% coverage. Both ends of pVir-KP0714 were absent from pOXA1_020030 but were highly homologous to another plasmid, pLAP2_020030 (GenBank accession no. CP028792), from WCHKP020030 (Figure 1; Appendix Figure 1). Multiple mobile genetic elements on these plasmids suggested that pVir-KP0714 was generated through genetic recombination between pOXA1_020030 and pLAP2_020030, which not only formed a novel virulence plasmid but also contributed to chromosomal integration of a 45-kb plasmid fragment from pLAP2_020030 (Figure 1; Appendix Figure 1). The 45-kb fragment that had not been integrated into pVir-KP0714 was divided into the upstream 30-kb fragment and a 15-kb genetic context containing bla_{SHV-12}, which were independently inserted into the chromosome. The 15-kb genetic context containing bla_{SHV-12} was flanked by several IS26 insertion sequences and harbored 3 other resistance genes, bla_{LAP-2}, *qnrS1*, and *aph(3')-Ia*, which exhibited 100% identity and 100% query coverage with the reference plasmid pLAP2_020030 (Figure 2; Appendix Figure 2).

However, we observed substantial structural changes in this chromosomal insertion fragment compared with pLAP2_020030 (Figure 2; Appendix Figure 2). The reversion and rearrangement of IS26-*aph(3')-Ia* provided an addition promoter P2 for bla_{SHV-12} (Figure 2; Appendix Figure 2). To determine the role of promoter P2 in CAZ/AVI resistance, we deleted P2 and the original promoter P1 of bla_{SHV-12} by using a pConj working vector-based genetic engineering approach

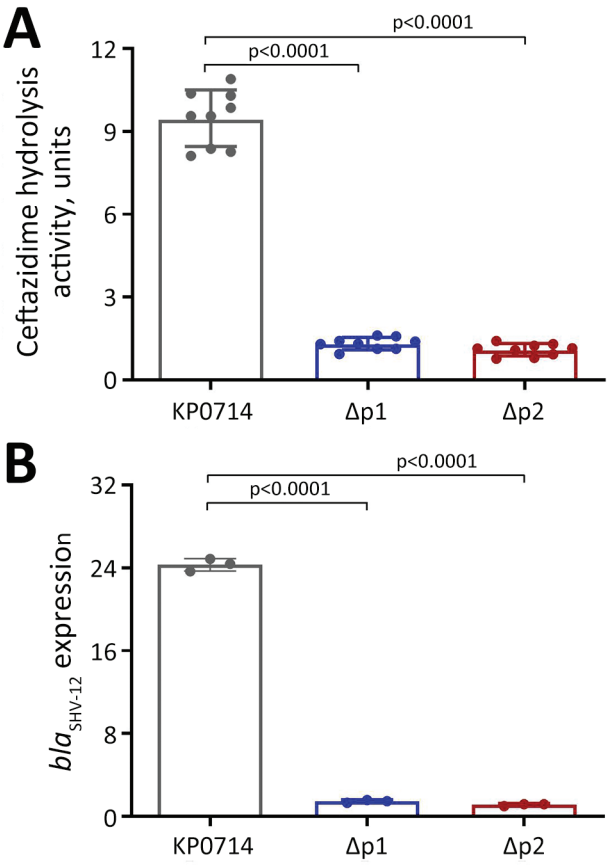

Figure 2. Overexpression of bla_{SHV-12} contributing to ceftazidime/avibactam resistance in *Klebsiella. pneumoniae* isolate KP0714 in study of ceftazidime/avibactam resistance in carbapenemase-producing *K. pneumoniae*. A) Relative bla_{SHV-12} expression level. B) Ceftazidime hydrolysis activity of different bla_{SHV-12} promoter deletion mutants. One unit of enzyme activity was defined as the amount of enzyme that hydrolyzed 1 nmol of substrate per min. Error bars indicate SDs. p values were computed by 1-way analysis of variance with Bonferroni correction.

(*6*). Deletion of P1 or P2 could completely restore KP0714 susceptibility to CAZ/AVI; CAZ/AVI MICs were 2 and 1 μg/mL, respectively (Appendix Table 1). The relative expression of bla_{SHV-12} in KP0714 was ≈20-fold higher than in ΔP1 and ΔP2 mutants (Figure 2; Appendix Figure 2). Similarly, the hydrolysis activity of ceftazidime in KP0714 was significantly higher than that of ΔP1 and ΔP2 mutants (p<0.0001). Those results demonstrated that CAZ/AVI resistance in KP0714 was attributed to overexpression of bla_{SHV-12} resulting from an additional promoter, and the original promoter P1 was also necessary for the biological function of P2.

Because a novel virulence plasmid pVir-KP0714 was formed through plasmid recombination, we determined the virulence potential of KP0714 by using a mouse infection model and human neutrophil

phagocytosis assay (*7*). As the hypervirulence control, we used the previously reported ST11 carbapenem-resistant hypervirulent *K. pneumoniae* strain CR-HvKP4 (*8*). We found no statistical difference regarding mouse survival and neutrophil phagocytosis between KP0714 and CR-HvKP4 (Figure 1; Appendix Figure 2), suggesting convergence of hypervirulence and CAZ/AVI resistance in KP0714. In contrast, mouse survival rates were significantly higher and human neutrophil phagocytosis rates were significantly lower for KP0714 and CR-HvKP4 at each time point when compared with virulence plasmid pVir-KP0714-curing KP0714 (PC-KP0714), demonstrating that KP0714 hypervirulence was attributed to acquisition of virulence plasmid pVir-KP0714.

In conclusion, KP0714 high-level resistance to carbapenems and CAZ/AVI, compensating for decreased carbapenem hydrolyzation activity of KPC variants (*5*,*9*), highlights a novel evolution pathway for development of CAZ/AVI resistance in epidemic ST11 KPC-KP, posing a threat to clinical antimicrobial therapy. Emerging CAZ/AVI-resistant and hypervirulent ST11 KPC-KP might be continuously evolving and warrants prospective monitoring.

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The complete genome sequences of KP0714 were deposited in the GenBank (accession nos. CP128191–5).

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Mrs. Cui is a researcher at Shanxi Provincial People's Hospital of Shanxi Medical University. Her research interests are epidemiology and antimicrobial-resistance mechanisms of carbapenem-resistant *Enterobacteriaceae*.

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