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Mutations in blaKPC-3 that emerged during ceftazidime-avibactam treatment of carbapenem-resistant K. pneumoniae infections encode novel KPC-3 variants that confer ceftazidime-avibactam resistance, restore carbapenem susceptibility, and function as extended-spectrum beta-lactamases

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Background: Ceftazidime-avibactam (C-A) is a novel β-lactam/β-lactamase inhibitor with activity against carbapenem-resistant Enterobacteriaceae (CRE) that produce *Klebsiella pneumoniae* carbapenemase (KPC). We encountered the emergence of C-A resistance during treatment of 5 patients with CR-*Klebsiella pneumoniae* infections (rate: ~10%; duration of treatment: 10-19 days). C-A resistant isolates exhibited restored carbapenem susceptibility (CS), and were identified as extended spectrum β-lactamase (ESBL)-producers by our clinical microbiology laboratory.

Material/methods: Mechanisms of C-A resistance were identified through whole-genome sequencing (WGS) of longitudinal *K. pneumoniae* clinical isolates. We validated bla_{KPC-3} mutations by: 1) bla_{KPC} disruptions in *K. pneumoniae* clinical isolates; 2) transfer of bla_{KPC}-containing plasmids into competent

E. coli; 3) cloning of bla_{KPC} genes into competent *E. coli*; 4) introduction of bla_{KPC-3} mutations by site-directed mutagenesis (SDM), and transfer into competent *E. coli*.

Results: WGS revealed mutations in plasmid-borne bla_{KPC-3} of C-A resistant isolates, which were not present in C-A susceptible baseline isolates. Mutations resulted in variant KPC-3 enzymes, and emerged independently in isolates of a novel sequence type (ST)-258 sublineage. KPC-3 variants included D179Y/T243M double substitution, D179Y, V240G, and insEL165-66. D179Y, alone or in combination with T243M, was the most common variant, identified in isolates from each patient. In one patient, four unique C-A resistant isolates with distinct bla_{KPC-3} mutations were recovered over time. T243M was not encountered alone in a clinical isolate, but only in combination with D179Y. D179Y and insEL165-166 mutations fall within the KPC-3 Ω-loop. Mutations encoding KPC-3 variants were shown to confer significantly increased C-A MICs and reduced carbapenem MICs. In rank order, the impact of variant KPC-3 enzymes on C-A and carbapenem MICs was D179Y/T243M >D179Y > V240G, T243M or insEL165-166. *E. coli* carrying mutant bla_{KPC-3} fulfilled the Clinical and Laboratory Standard Institute's criteria for presence of ESBL.

Conclusions: These are the first reports of C-A resistance emerging in clinical CRE isolates during treatment. Based on epidemiologic and WGS data, infections due to C-A resistant isolates did not reflect nosocomial transmission or acquisition of an institutional clone. We confirmed that 5 bla_{KPC-3} mutations that arose independently in different *K. pneumoniae* clinical isolates directly caused C-A resistance and resulted in variant KPC-3 enzymes that functioned as ESBLs, rather than carbapenemases. The clinical implications of restored CS and the stability of bla_{KPC-3} mutations are currently under investigation. We are also investigating if the unique ST258 sublineage at our center is particularly susceptible to bla_{KPC-3} mutations and C-A resistance.