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

Minh-Hong Nguyen1, Ryan Shields2, Shaoji Cheng2, Liang Chen3, Barry Kreiswirth4, Cornelius Clancy*2

1University of Pittsburgh; Infectious Diseases
2University of Pittsburgh
3Rutgers University New Jersey Medical School
4New Jersey Medical School, Rutgers The State University of New Jersey; Public Health Research Institute Centre

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 blaKPC-3 mutations by: 1) blaKPC disruptions in K. pneumoniae clinical isolates; 2) transfer of blaKPC-containing plasmids into competent
E. coli; 3) cloning of bla\textsubscript{KPC} genes into competent E. coli; 4) introduction of bla\textsubscript{KPC-3} mutations by site-directed mutagenesis (SDM), and transfer into competent E. coli.

**Results:** WGS revealed mutations in plasmid-borne bla\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{KPC-3} mutations are currently under investigation. We are also investigating if the unique ST258 sublineage at our center is particularly susceptible to bla\textsubscript{KPC-3} mutations and C-A resistance.