

O0929 Ceftazidime-avibactam resistance due to KPC omega-loop mutations is common in clinical *K. pneumoniae* isolates from a novel ST258, clade II sublineage, which are not hypermutators

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Background: Ceftazidime-avibactam (C-A) achieves better outcomes in treating carbapenem-resistant Enterobacteriaceae (CRE) infections than other antimicrobial regimens. We previously reported the emergence of C-A resistant (R) CR-*K. pneumoniae* (CRKP) in 4 patients (pts) treated with the drug. We now report on C-A R mechanisms and genomic epidemiology for serial isolates from 8 CR-Kp-infected pts.

Materials/methods: Isolates underwent whole genome sequencing (MiSeq). A phylogenetic tree was constructed based on concatenated core genome SNPs. *KPC* genes were PCR-amplified and sequenced. Mutant *KPC* were validated as R determinants by site-directed mutagenesis, and transformation of *E. coli*. Mutational frequency was assessed by assaying 10¹⁰-10¹¹ CRKP cells (13 clinical isolates) in presence or absence of drug for 24-36 hr on solid agar *in vitro*.

Results: C-A R emerged in 10% (8/77) and 14% (8/59) of pts treated for CRE and CRKP infections, respectively. There were no epidemiologic links between cases in which R arose, and there was no evidence for hospital transmission. In each case, R was caused by mutations in *KPC*-3 (22%, 8/37 vs. 0%, 0/19, for *KPC*-2; *p*=0.04). The Ω-loop D179Y mutation, alone or in combination with other mutations, caused R in 88% (7/8) of pts. Meropenem MCs decreased ≥4-fold in isolates expressing mutant *KPC*-3 enzymes. Baseline and post-exposure isolates from 88% (7/8) of pts clustered within a novel ST258, clade II CRKP sublineage not previously encountered at our center or others. Isolates differed by 20 median core genome SNPs. Isolates from the remaining pt clustered independently from other ST258, clade II isolates, differing by ≥157 SNPs. Mutational frequency rates were ≤10⁻⁷ on exposure to rifampin and other antimicrobials/stressors, and did not differ significantly between isolates of different ST258 clades or sublineages.

Conclusions: Isolates from a novel ST258, clade II CRKP sublineage are particularly susceptible to developing C-A R due to *KPC* mutations, but they are not hypermutators *in vitro*. Our experience suggests that the novel sublineage may be prone to R *in vivo*, or to cryptic nosocomial transmission.