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Resistance to Ceftazidime-Avibactam in *Klebsiella pneumoniae* Due to Porin Mutations and the Increased Expression of KPC-3

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We reported the first clinical case of a ceftazidime-avibactam resistant KPC-3-producing *Klebsiella pneumoniae* (1), from a patient with no history of ceftazidime-avibactam therapy. We now present data documenting mechanisms of ceftazidime-avibactam resistance in this isolate. Whole-genome sequencing (WGS) was performed on two isolates: KP1245 (ceftazidime-avibactam MIC, 4 μg/ml; from blood on hospital day 1; referred to as isolate 1 in our previous report [1]) and KP1244 (ceftazidime-avibactam MIC, 32 μg/ml; from blood on hospital day 2; referred to as isolate 2 in our previous report [2]), using MiSeq (Illumina, San Diego, CA) and PacBio RSII (Menlo Park, CA) systems (2). The i*n silico* multilocus sequence type (ST) was ST258. Single nucleotide polymorphism (SNP) analysis revealed 17 SNPs between KP1245 and KP1244, indicating that the isolates were related but that significant diversity existed in this patient (2). Nonsynonymous mutations are shown in Table 1; the most striking of these is in the OmpK36 porin gene. KP1244 contained a missense mutation predicted to encode a T333N mutation. Both isolates also harbored a mutation predicted to encode R191L in OmpK36 and had a nonfunctional OmpK35, due to a frameshift mutation that truncated the protein at amino acid 42, common to *K. pneumoniae* ST258 (3). Association between mutations in *ompK36* and elevated ceftazidime-avibactam MICs has been shown previously (4). However, T333N, found in one of the β-sheet domains of the OmpK36 subunit, has not been described in *K. pneumoniae*; as such, further validation is required to confirm the role of the OmpK36 mutation in this isolate's ceftazidime-avibactam resistance phenotype.

Both isolates harbored $bla_{\text{SHV-11}}$ on the chromosome and $bla_{\text{KPC-3}}$ and $bla_{\text{SHV-12}}$ on the sole plasmid. The plasmid was a 205-kb plasmid, pUCLAKPC with an IncX3 origin of replication. Two copies of the $bla_{\text{KPC-3}}$ Tn*4401d* transposon were in both isolates (5). Plasmid copy number was estimated, from WGS read coverage data and real-time PCR comparing efficiency-corrected cycle thresholds (C_T) between $bla_{\text{KPC-3}}$ and the 16S rRNA genes (6), to be 4 for both isolates. Real-time quantitative PCR from 3 experiments demonstrated that the $bla_{\text{KPC-3}}$ expression of KP1244 was 3.8- $\pm$ 0.2-fold that of KP1245. This correlated with meropenem MICs of 512 μg/ml versus 16 μg/ml. Of note, no mutations in the Ω-loop were found in $bla_{\text{KPC-3}}$ genes.

Surveillance studies continue to document very low rates of resistance to ceftazidime-avibactam among the *Enterobacteriaceae* (7). However, testing of ceftazidime-avibactam resistance is not common in microbiology laboratories, and resistance may be underappreciated. While our patient had no exposure to ceftazidime-avibactam, she was treated with meropenem and cefepime (1), providing the selective pressure required for mutation to OmpK36. Emergence of mutations to OmpK36 during treatment with carbapenems is relatively common (8, 9). While OmpK35 and OmpK36 are not thought

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TABLE 1 Nonsynonymous mutations identified between KP1244 (ceftazidime-avibactam resistant) and KP1245 (susceptible)

Predicted protein (gene)	Base variation (KP1244:KP1245)	Predicted amino acid variation (KP1244:KP1245; amino acid position)
Outer membrane porin C (*ompK36*)	T:G	N:T; 333
DNA binding capsular synthesis gene (*rcsB*)	G:A	G:S; 184
Sensory kinase gene (*barA*)	T:G	D:A; 471
Nitriloacetate monooxygenase component A (*ntaA*)	A:G	T:A; 290
Protein of unknown function (*slyX*)	T:C	C:R; 46
Ferrichrome-iron receptor (*fhuA*)	A:T	Q:T; 583
Cytosol aminopeptidase (*pepA*)	C:T	M:I; 106

to be the primary pathway for avibactam into *K. pneumoniae* (10), combination of this with increased KPC expression is thought to have led to the resistance phenotype in KP1244. Castanheira and colleagues have reported a KPC-2-expressing isolate with ceftazidime-avibactam resistance, which was attributed to decreased expression of *ompK36* and a premature stop codon in *ompK35* (11). These authors did not evaluate KPC expression, so it is possible that this isolate, similar to ours, had heightened KPC activity which, when combined with reduced entry of ceftazidime and avibactam into the cell, resulted in the resistance phenotype.

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