Mutations in *bla*$_{KPC-3}$ during ceftazidime-avibactam treatment of carbapenem-resistant *Klebsiella pneumoniae* infections

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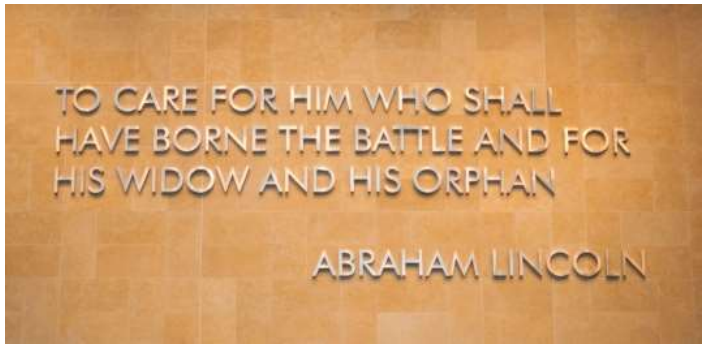


University of Pittsburgh

VAPHS
VA Pittsburgh Healthcare System

Disclosures and conflicts of interest

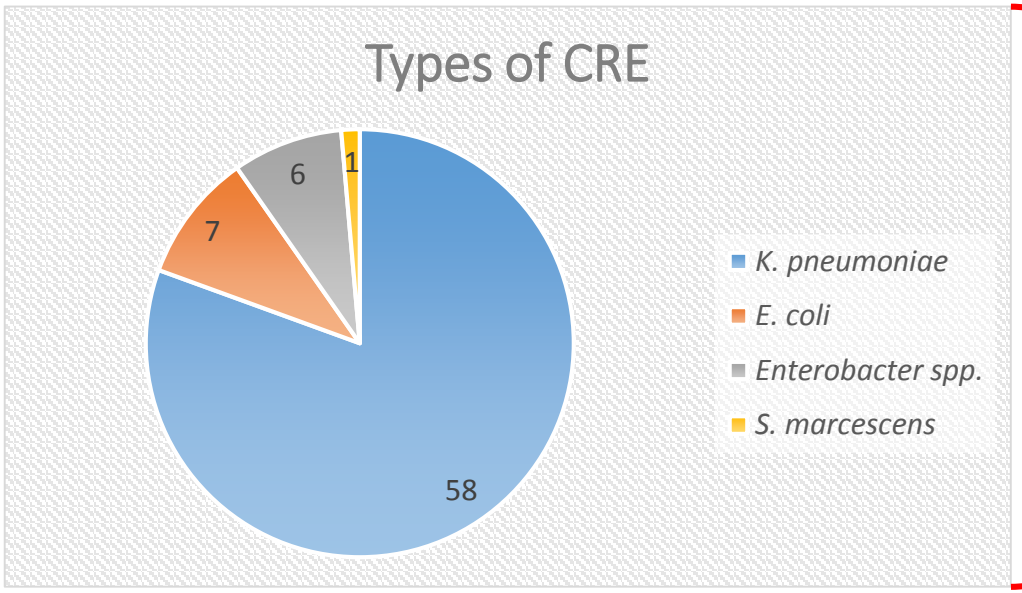
- Laboratory funding from NIH and VA grants
  - Candida, CRE, lung microbiome, Legionella
- Astellas, Pfizer, MSD, CSL-Behring, Cidara support for investigator-initiated research projects
- Astellas, MSD, Cidara, Medicines Company for speaking and advisory boards
- Site PI, T2 Biosystems clinical trials
- No financial holdings

Objectives

- To describe our experience with ceftazidime-avibactam against CRE infections at UPMC
  - Toxicity
  - Clinical outcomes
  - Emergence of resistance
- To identify and validate ceftazidime-avibactam resistance mechanisms

Results

- 72 patients treated with ceftazidime-avibactam (≥ 3 d) for CRE infections between April 2015 and February 2017



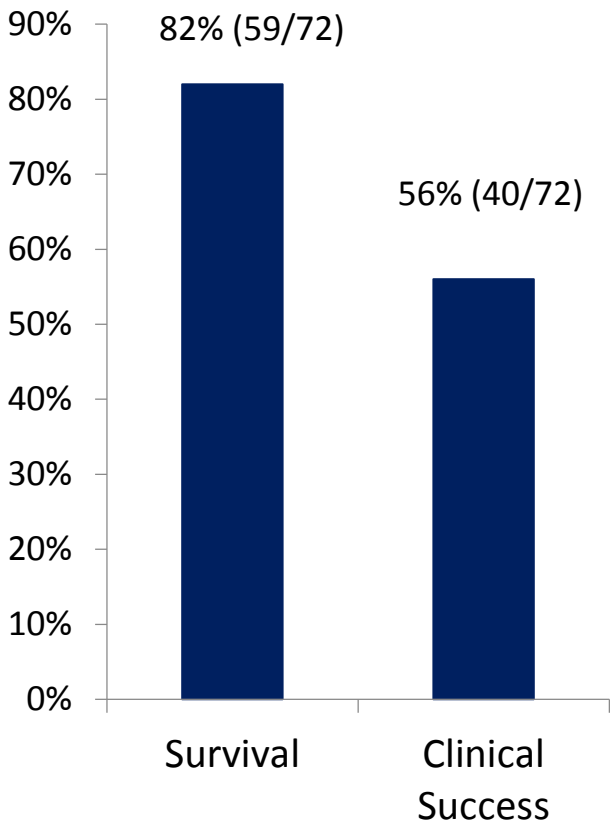
78% KPC producers

- Ceftazidime-avibactam was well-tolerated
  - Acute kidney injury (end of treatment): 15% (10/66)

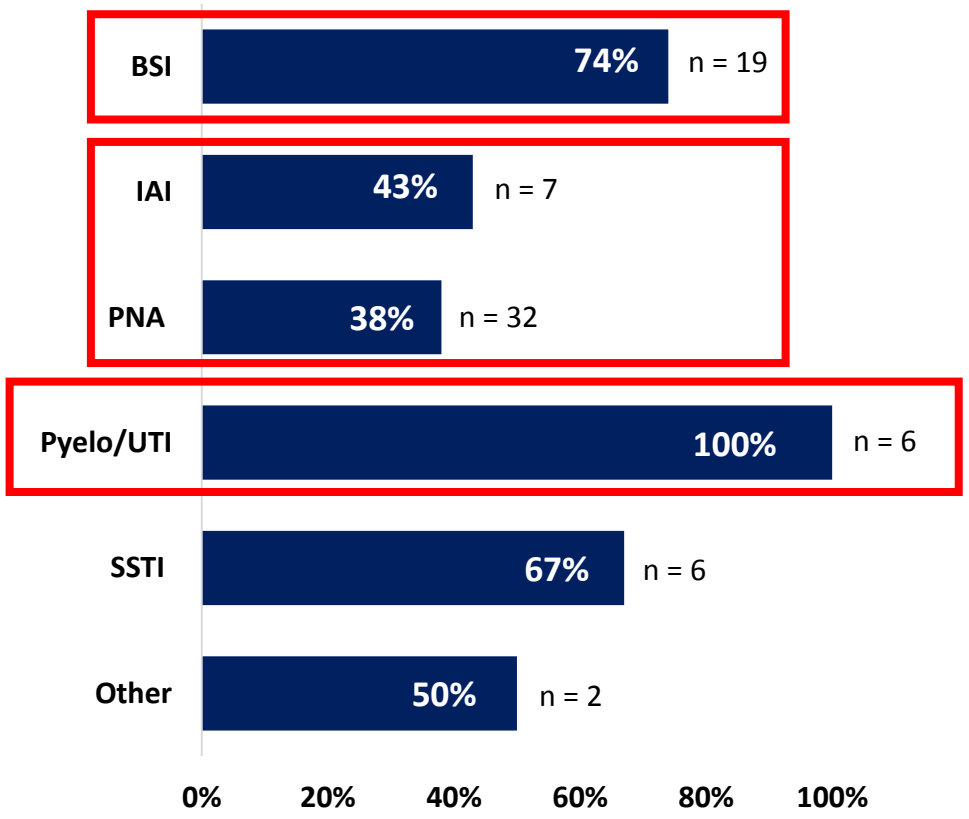
Colistin: 57%
Aminoglycosides: 44%

Clinical outcomes

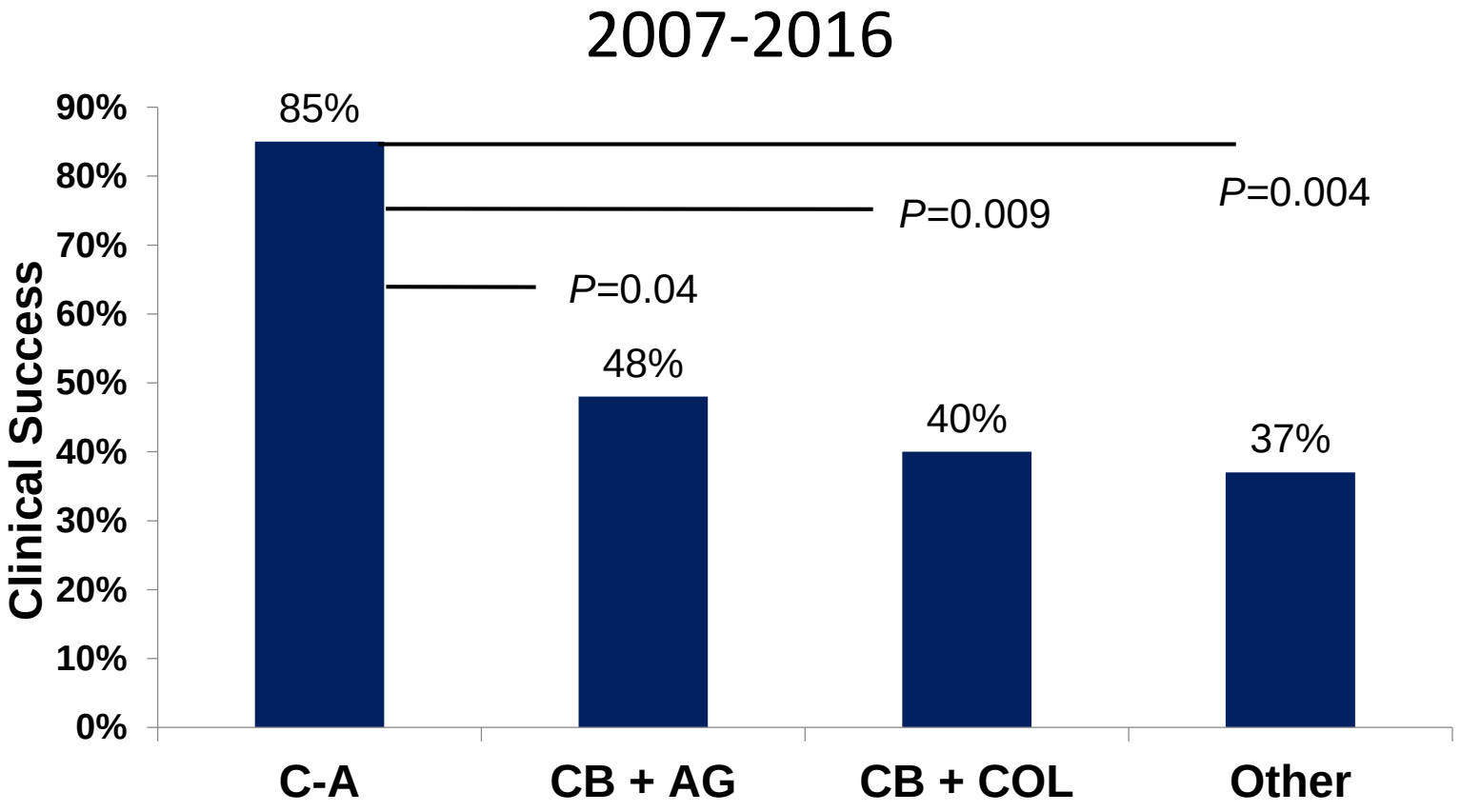
30 d Outcomes



Success by Disease Type



CR-Kp bacteremia: Outcomes by antibiotic regimen



C/A was independently associated with clinical success (OR=8.64; 95% CI: 1.61 – 46.39; *P*=0.01)

No significant differences between groups in underlying diseases, severity of illness, and strain characteristics

Risk factors for C-A failure

Factor	Success (n=40)	Failure (n=32)	*P*-value
Median age, years (range)	64.5 (19 – 91)	60.5 (26 – 79)	0.39
Transplant recipient, n (%)	9 (22)	8 (25)	0.80
Median Charlson Score (range)	5 (0 – 10)	4.5 (0 – 10)	0.81
Renal replacement therapy, n (%)	**3 (8)**	**12 (38)**	**0.002**
Median SAPS II score (range)	40 (18 – 79)	43 (17 – 81)	0.06
Median SOFA score (range)	**5 (1 – 20)**	**8 (0 – 20)**	**0.002**
CRE pneumonia, n (%)	**12 (30)**	**20 (63)**	**0.009**
Receipt of combination therapy, n (%)	8 (20)	7 (22)	0.85

38% of patients

Ceftazidime-avibactam resistance

12% of patients (9/72) treated for CRE infections developed ceftazidime-avibactam resistance

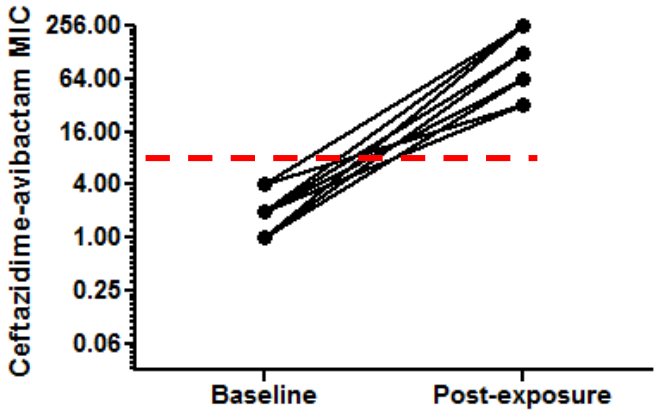
16% of patients (9/58) treated for CR-Kp infections developed ceftazidime-avibactam resistance

Patient	Days of C-A	Infection Type	Location	Treatment regimen	RRT*	Outcome at 30 days
1	10	PNA	MICU	Monotherapy	No	Failure
2	19	IAI	SICU	Monotherapy	CRRT	Failure
3	15	PNA	SICU	Monotherapy	No	Success w/ relapse
4	15	PNA	CTICU	+ inhaled gent	CRRT	Failure
5	15	PNA	MICU	Monotherapy	HD	Failure
6	7	PNA	MICU	Monotherapy	No	Failure
7	15	PNA	6FG	Monotherapy	No	Success w/ relapse
8	25	PNA	10G	Monotherapy	HD	Failure
9	31	PNA	CTICU	+ inhaled/IV gent	CRRT	Failure

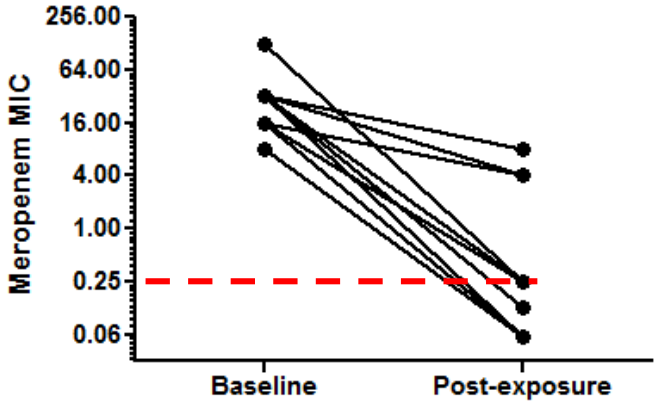
* Independent risk factor for resistance (OR: 11.70, 95% CI: 1.79 – 76.0; *P*=0.003)

Ceftazidime-avibactam resistance

Ceftazidime-avibactam MICs



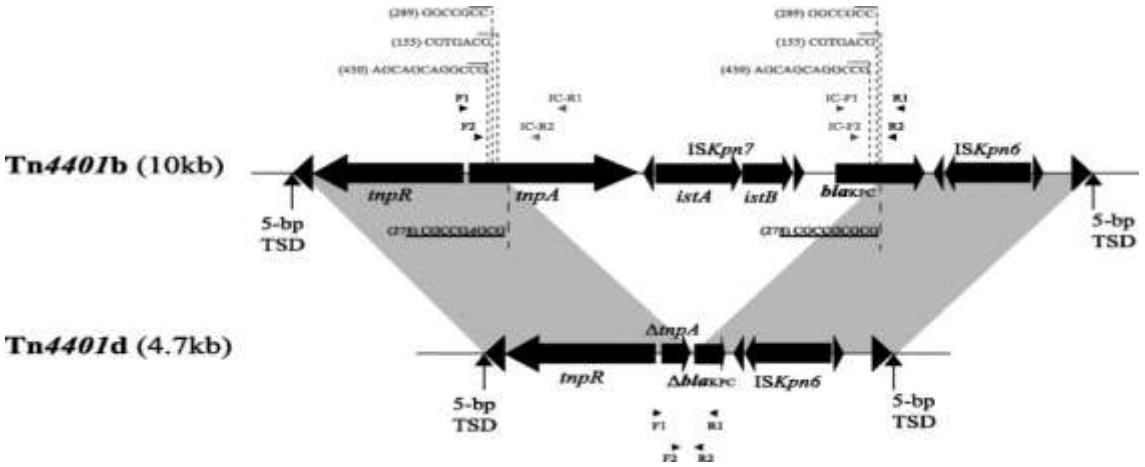
Meropenem MICs



Patient	Baseline KPC variant	New KPC variant(s)
1	KPC-3	D179Y, T243M
2	KPC-3	V240G and D179Y
3	KPC-3	D179Y
4	KPC-3	A177E, D179Y
5	KPC-3	D179Y
6	KPC-3	D179Y
7	KPC-3	168 – 169 EL del and D179Y
8	KPC-3	169 – 170 EL ins
9	KPC-3	D179Y

Whole genome sequencing

- *bla*$_{KPC}$ genes were found on Δ*Tn1331*-Tn*4401*d
  - Within an IncFIA pBK30683 plasmid



Whole genome sequencing

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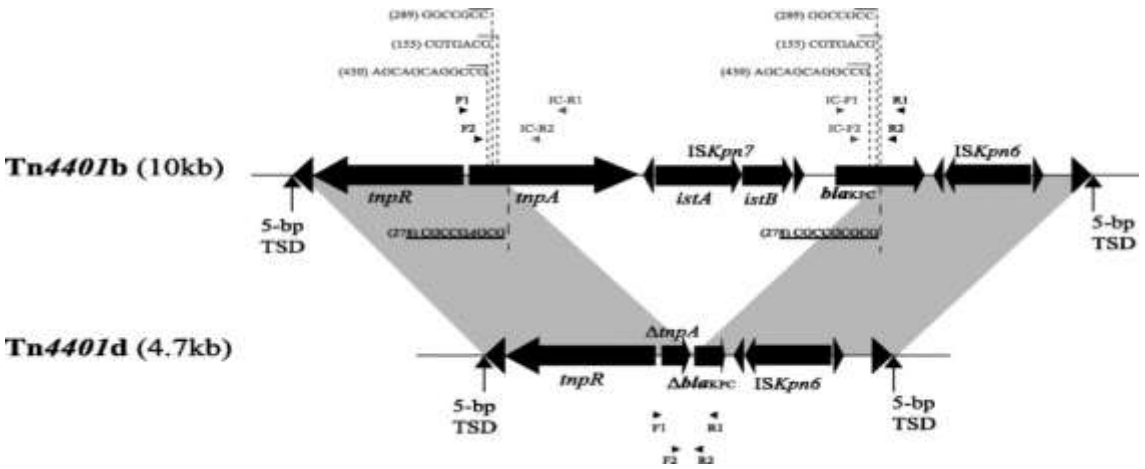
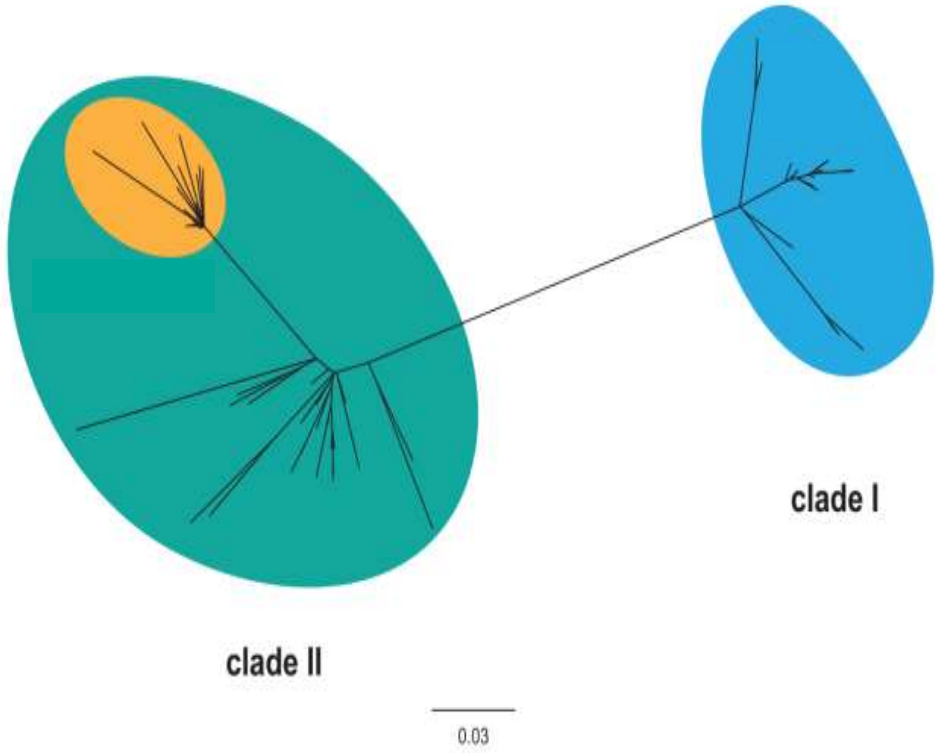


- Novel ST258 sublineage

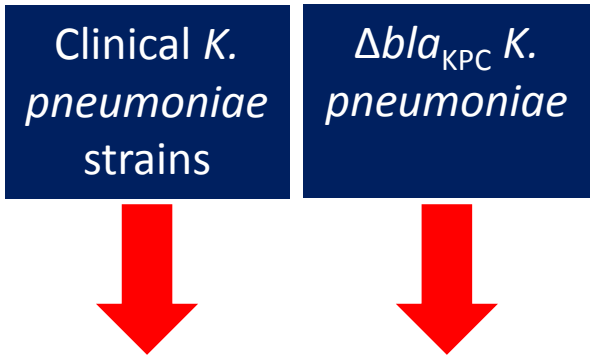


Validation of *bla*$_{KPC-3}$ mutations

Clinical *K. pneumoniae* strains

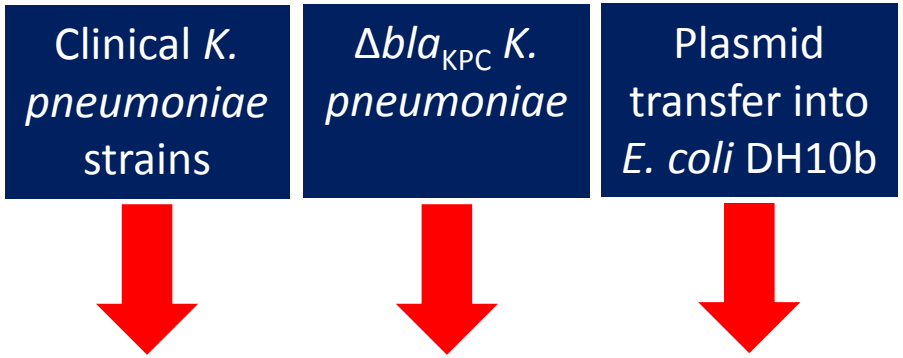
KPC variant	Baseline MIC (µg/mL)	
	C/A	MER
KPC-3	2	32
D179Y, T243M	256	0.25
D179Y	128	0.25
V240G	32	8
168-169EL Ins	32	8

Validation of *bla*$_{KPC-3}$ mutations



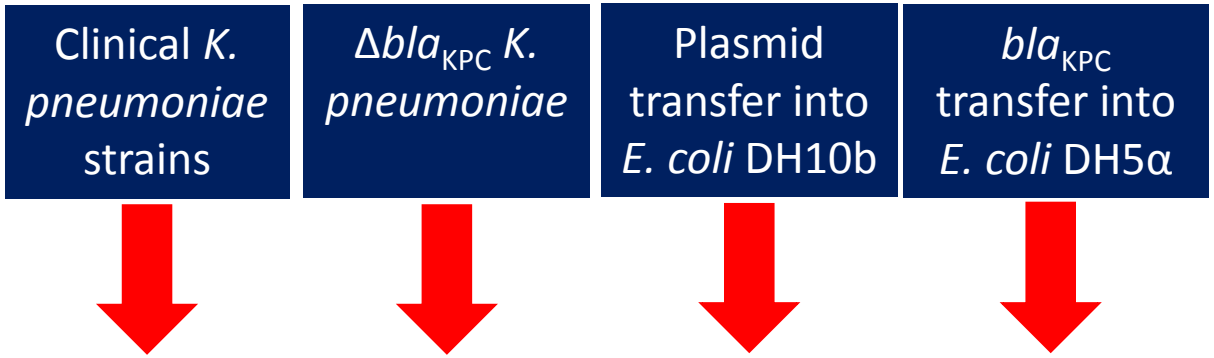
KPC variant	Baseline MIC (µg/mL)		Δ*kpc* MIC (µg/mL)	
	C/A	MER	C/A	MER
KPC-3	**2**	**32**	**1**	**0.125**
D179Y, T243M	**256**	**0.25**	**≤0.25**	**0.125**
D179Y	**128**	**0.25**	**≤0.25**	**0.125**
V240G	**32**	**8**	**0.5**	**0.125**
168-169EL Ins	**32**	**8**	**0.5**	**0.125**

Validation of *bla*$_{KPC-3}$ mutations



KPC variant	Baseline MIC (µg/mL)		Δ*kpc* MIC (µg/mL)		pBK30683 MIC (µg/mL)	
	C/A	MER	C/A	MER	C/A	MER
KPC-3	**2**	**32**	**1**	**0.125**	**0.5**	**4**
D179Y, T243M	**256**	**0.25**	**≤0.25**	**0.125**	**64**	**0.06**
D179Y	**128**	**0.25**	**≤0.25**	**0.125**	**16**	**0.06**
V240G	**32**	**8**	**0.5**	**0.125**	**4**	**0.125**
168-169EL Ins	**32**	**8**	**0.5**	**0.125**	**4**	**1**

Validation of *bla*$_{KPC-3}$ mutations



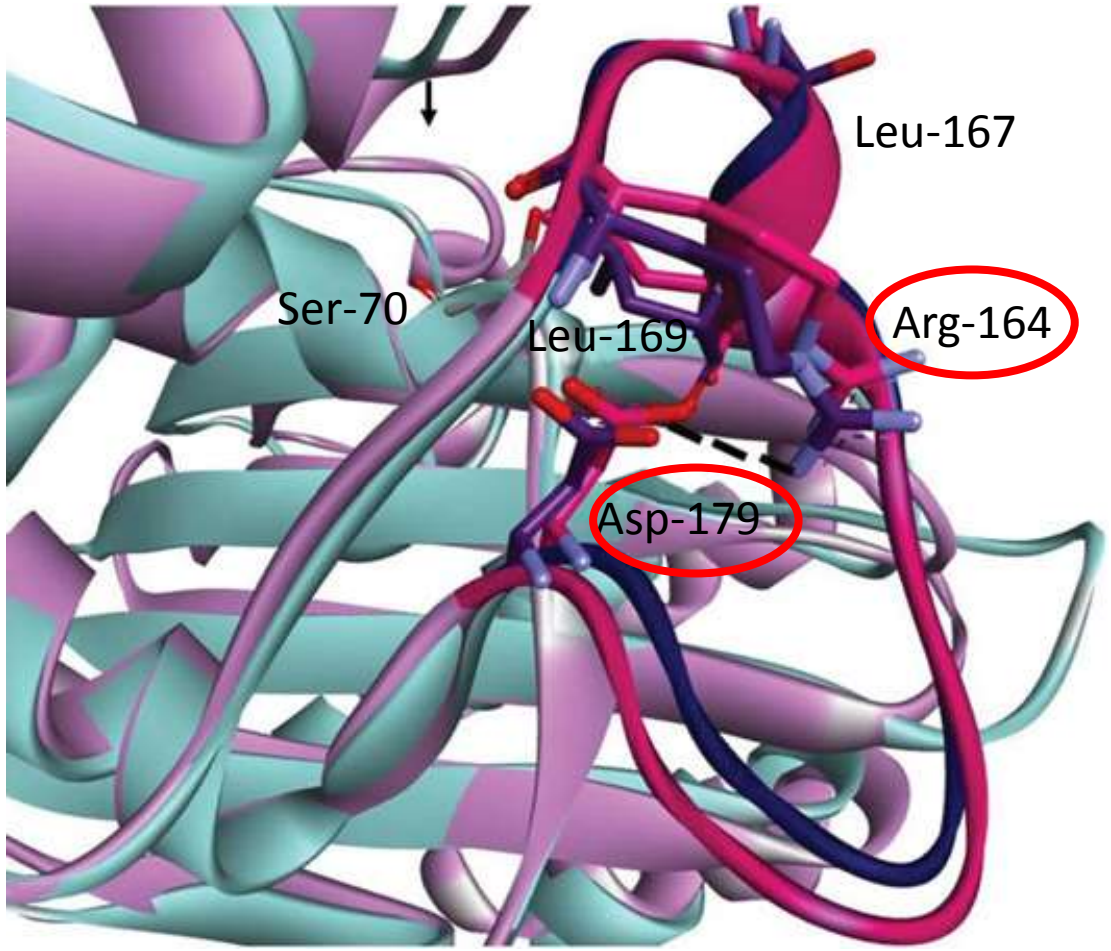
KPC variant	Baseline MIC (µg/mL)		Δ*kpc* MIC (µg/mL)		pBK30683 MIC (µg/mL)		pET30a MIC (µg/mL)	
	C/A	MER	C/A	MER	C/A	MER	C/A	MER
KPC-3	2	32	1	0.125	0.5	4	0.5	4
D179Y, T243M	256	0.25	≤0.25	0.125	64	0.06	64	0.06
D179Y	128	0.25	≤0.25	0.125	16	0.06	8	0.06
V240G	32	8	0.5	0.125	4	0.125	2	1
168-169EL Ins	32	8	0.5	0.125	4	1	2	1

Validation of *bla*$_{KPC-3}$ mutations

Clinical *K. pneumoniae* strains	Δ*bla*$_{KPC}$ *K. pneumoniae*	Plasmid transfer into *E. coli* DH10b	*bla*$_{KPC}$ transfer into *E. coli* DH5α	*bla*$_{KPC}$ S.D.M. transfer into *E. coli* DH5α

KPC variant	Baseline MIC (µg/mL)		Δ*kpc* MIC (µg/mL)		pBK30683 MIC (µg/mL)		pET30a MIC (µg/mL)		pET30a MIC (µg/mL)	
	C/A	MER	C/A	MER	C/A	MER	C/A	MER	C/A	MER
KPC-3	2	32	1	0.125	0.5	4	0.5	4	0.5	4
D179Y, T243M	256	0.25	≤0.25	0.125	64	0.06	64	0.06	16	0.06
D179Y	128	0.25	≤0.25	0.125	16	0.06	8	0.06	8	0.06
V240G	32	8	0.5	0.125	4	0.125	2	1	8	0.125
168-169EL Ins	32	8	0.5	0.125	4	1	2	1	4	0.125

KPC Ω-loop mutations



- Amino acid positions 164 to 179
- Substitutions in this region result in enhanced ceftazidime affinity
  - May prevent binding of avibactam
- Previously identified among KPC-2 producing *K. pneumoniae* through *in vitro* selection

Livermore DM, et al. *AAC* 2015;59:5324
Winkler ML, et al. *JAC* 2015;70:2279

Winkler ML, et al. *JAC* 2015;70:2279

Conclusions

- Ceftazidime-avibactam is an important addition to the armamentarium against CRE infections
  - Outcomes at least equivalent to previous outcomes with 2 active agents
    - May be superior *vs.* CRE bacteremia
  - Well-tolerated
- Emergence of resistance is concerning
- Resistance is mediated by KPC-3 mutations, particularly within Ω-loop
  - Associated with reduced carbapenem MICs
  - May be misidentified as ESBLs

Unresolved issues

- What is the role of combination therapy with cetazidime-avibactam in improving outcomes and limiting resistance?
- What is the clinical significance of reduced carbapenem MICs?
- How widespread is the emergence of ceftazidime-avibactam resistance?
  - Are there issues unique to UPMC?
  - Are ST258 or the unique ST258 sublineage pre-disposed?
  - Is KPC-3 more susceptible?
  - Is nosocomial spread playing a role in emergence at UPMC?

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