Evaluation of Meropenem-Vaborbactam Susceptibility and Underlying Resistance Mechanisms among Clinical KPC-producing *Klebsiella pneumoniae*

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Background

- Meropenem-vaborbactam (MV) is the first carbapenem/β-lactamase inhibitor combination developed to restore meropenem susceptibility against KPC-producing carbapenem-resistant *Enterobacterales* (CRE).
- Vaborbactam (VAB) potently inhibits Ambler class A and C β-lactamases by reversible covalent binding of boronate to serine side chains of β-lactamases.
- Resistance to MV in non-metallo-β-lactamase (MBL) producing *Klebsiella pneumoniae* (KP) isolates has been described but remains rare.
- We sought to identify the major molecular mechanisms associated with MV resistance in KPC-producing *KP* (KPC-*KP*) isolates.

Methods

- Clinical isolates with elevated MV minimum inhibitory concentrations (MICs) were identified by the consult service.
- Additional clinical isolates with mutations in *ompK35* or *ompK36* genes were selected from a historic database.
- Isolates with MBL or OXA-48-like genes were excluded.
- Controls were comprised of MV susceptible KPC-*KP* isolates.
- MICs determination was done using Sensititre automated broth microdilution (BMD) according to CLSI.
- VAB and avibactam concentrations were held at 8 µg/ml and 4 µg/ml, respectively.
- Whole genome sequencing (WGS) was performed on all isolates. Genome libraries were prepared using Illumina Nextera XT and sequencing was performed on MiSeq and MinION.

Results

- A total 119 KPC-*KP* isolates were included.
- All isolates were resistant to meropenem.
- 21 KPC-*KP* with elevated MV MICs were identified.
- All MV resistant isolates harbored mutations in *ompK36* genes.
- Glycine/aspartate (GD 134-135) insertion, premature stop codon in *ompK36* genes, and concomitantly elevated bla_{KPC} copy number were predominant among MV resistant isolates.
- No insertion elements in *ompK36* gene promoter region were found.
- Two MV resistant isolates exhibited unique mutations in bla_{KPC} and *envZ* genes.

Results

Table. Whole genome sequencing of KPC-*KP* isolates with elevated MV MICs

Strain	Year	β-lactamase genes	MLST	Outer membrane porin			Estimated bla_{KPC} copy	MIC (µg/ml)				Other mutations
				ompK35	*ompK36*	*ompK37*		MEM	MVB	CFD	CZA	
1	2012	bla_{KPC-2}, bla_{SHV-12}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	1.79	>64	2/8	0.5	1/4	bla_{SHV} (G238S; E240K; L35Q)
2	2012	bla_{KPC-2}, $bla_{SHV-182}$, bla_{TEM-1A}, bla_{OXA-9}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	5.67	>64	>16/8	8	2/4	bla_{SHV} (G238S; E240K; L35Q)
3	2018	bla_{KPC-2}, bla_{SHV-12}^{α}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	5.09	>64	>16/8	2	8/4	envZ (A225V)
4	2014	bla_{KPC-2}, bla_{SHV-12}	ST258	FS 42insG, aa89*	GD	K251*	0.97	>64	>16/8	0.5	1/4	bla_{SHV} (G238S; E240K; L35Q)
5	2019	bla_{SHV-99}, bla_{CMY-2}	ST323	WT	aa64*	No FS or stop codons	N/A	>64	8/8	4	4/4	↑bla_{CMY-2} copy 15X; ramR(K194*)
6	2019	bla_{KPC-2}, bla_{SHV-12}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	1.67	>64	>16/8	2	8/4	bla_{SHV} (G238S; E240K; L35Q)
7	2018	bla_{KPC-2}, $bla_{SHV-182}$,$bla_{TEM-122}$, bla_{OXA-9}	ST258	FS 42insG, aa89*	GD	No FS or stop codons	1.47	>64	4/8	0.24	1/4	No mutations
8	2013	bla_{KPC-2}, $bla_{SHV-182}$, bla_{TEM-1A}, bla_{OXA-9}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	11.63	>64	>16/8	0.12	1/4	bla_{SHV} (L35Q)
9	2014	bla_{KPC-2}, bla_{SHV-12}, bla_{TEM-1A}, bla_{OXA-9}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	0.86	>64	4/8	4	1/4	bla_{SHV} (G238S; E240K; L35Q)
10	2019	bla_{KPC-2}, $bla_{SHV-182}$	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	1.29	>64	8/8	4	0.12/4	bla_{SHV} (L35Q)
11	2018	$bla_{OXA-232}$, bla_{OXA-1}, $bla_{SHV-106}$, bla_{TEM-1A}, $bla_{CTX-M-15}$	ST2096	WT	GD, L312*	No FS or stop codons	N/A	>64	>16/8	1	4/4	ramR (K194*)
12	2013	bla_{KPC-2}, $bla_{SHV-182}$ bla_{TEM-1A}, bla_{OXA-9}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	1.42	>64	2/8	0.25	1/4	ramR (FS 111-113insP and aa159*); blaSHV(L35Q)
13	2013	bla_{KPC-2}, $bla_{SHV-182}$	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	5.18	>64	>16/8	0.12	1/4	bla_{SHV} (L35Q)
14	2018	bla_{KPC-2}, bla_{SHV-12}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	8.14	>64	>16/8	1	4/4	bla_{KPC} (S274N); bla_{SHV} (G238S;E240K;L35Q)
15	2013	bla_{KPC-2}, bla_{SHV-12}, bla_{TEM-1A}, bla_{OXA-9}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	1.31	>64	>16/8	0.5	0.5/4	bla_{SHV} (G238S; E240K; L35Q)
16	2013	bla_{KPC-2}, bla_{SHV-12}, bla_{TEM-1A}, bla_{OXA-9}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	2.43	>64	8/8	8	4/4	bla_{SHV} (G238S; E240K; L35Q)
17	2018	bla_{KPC-2}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	1.6	>64	8/8	0.06	4/4	bla_{SHV} (L35Q)
18	2013	bla_{KPC-2}, bla_{SHV-12}, bla_{TEM-1A}, bla_{OXA-9}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	2	>64	2/8	2	0.25/4	bla_{SHV} (G238S; E240K; L35Q)
19	2015	bla_{KPC-2}, bla_{SHV-12}	ST258	FS 42insG, aa89*	WT	FS: G240insP, K251*	0.76	>64	>16/8	4	1.5/4	bla_{SHV} (G238S; E240K; L35Q)
20	2018	bla_{KPC-2}, bla_{SHV-12}	ST258	FS 42insG, aa89*	GD	FS: G240insP, K251*	2.07	>64	>16/8	N/A	4/4	bla_{SHV} (G238S;E240K;L35Q)
21	2015	bla_{KPC-4}, $bla_{SHV-164}$	ST76	aa64*	aa83*	No FS or stop codons	41.87	>64	8/8	16	16/4	ramR (K194*); KPC-4 (V240G; P104R; W105G)

Conclusion

- MV resistant KPC-*KP* isolates were reliably analyzed using WGS to reveal the contribution of *omp* gene mutations and bla_{KPC} copy number to this phenotype.
- Elevated MV MICs were additionally recognized among clinical isolates from a historic database predating MV availability.
- CZA appears to retain activity against these isolates.
- In the absence of MBL production, caution remains warranted with the use of MV empirically against KPC-*KP* due to non-β-lactamase mediated resistance mechanisms.

Key – Abbreviations

α: Truncated at nodes 14 and 76, partial genotype consistent with bla_{SHV-12}
WT: Wild type
*: Premature stop codon
aa: amino acid
GD: Duplication of Glycine (G134) and Aspartate (D135)
FS: Frameshift ins: insertion
MEM: meropenem; MVB: meropenem-vaborbactam; CZA: ceftazidime-avibactam
CFD: cefiderocol

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