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Impact of porin deletions on the *in vitro* antimicrobial activity of cefepime-taniborbactam (formerly VNRX-5133) in *Klebsiella pneumoniae*

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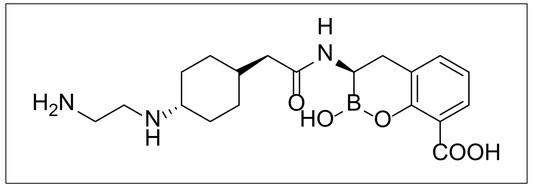
Background

Taniborbactam (formerly VNRX-5133) is an injectable cyclic boronate β-lactamase inhibitor that restores antibacterial activity of cefepime against multi-drug resistant *Enterobacterales* and *P. aeruginosa* producing any class of β-lactamases including both serine- and metallo-β-lactamases. A Phase 3 trial of the cefepime-taniborbactam combination is currently underway. Herein, we report the mechanisms of resistance to cefepime-taniborbactam in *Klebsiella pneumoniae*, and evaluate the impact of deletion of porin genes (*ompK35* and *ompK36*) and/or the master regulator gene *ramA* on the activity of cefepime-taniborbactam in *K. pneumoniae* isogenic strains.

Structure of taniborbactam



Materials/Methods

Minimum inhibitory concentrations (MICs) were determined by broth micro-dilution following CLSI methods. Isolation of mutants less susceptible to cefepime-taniborbactam was performed by selection of *K. pneumoniae* strains producing NDM-1. Whole genome sequencing was used to identify the mutation sites. *K. pneumoniae* isogenic strains lacking OmpK35, OmpK36 and/or RamA, with or without expression of KPC-3 β-lactamase were obtained from Kemyth Biotech.

Selection of less susceptible mutants by cefepime-taniborbactam

Mutant selection of *K. pneumoniae* strains expressing NDM-1 (isolated at the Royal Hospital of Muscat, Sultanate of Oman)

Strains	ST type	β-lactamase	Agar MIC Cefepime + 4 µg/mL taniborbactam	Mutant selection condition: cefepime + taniborbactam Concentrations	Number of colonies selected	Number of selected colonies	Frequency of resistance
***K. pneumoniae* OMAN 8**	ST101	NDM-1, CTX-M-15, OXA-1, TEM-1	4	16 + 4	1.01×10^{11}	5	**4.95×10^{-11}**
***K. pneumoniae* OMAN 19**	ST15	NDM-1, CTX-M-15, SHV-12, OXA-1,	4	16 + 4	0.90×10^{11}	3	**3.33×10^{-11}**

MICs of isolated mutants and genome sequence analysis

Strains	Cefepime MIC	Cefepime MIC + 4 µg/mL taniborbactam	Meropenem MIC	Meropenem MIC + 4 µg/mL taniborbactam	β-lactamases	NDM-1 coverage*	OmpK35	OmpK36	RamR	PBP3
OMAN 8	128	1	32	<0.06	-	1.2	-	-	-	-
OMAN 8 mutant #1	256	16	32	<0.06	No SNP	10.7	No SNP	No SNP	No SNP	No SNP
OMAN 19	256	2	32	<0.06	-	1.3	W230stop	-	-	-
OMAN 19 mutant #1	512	32	16	0.12	No SNP	1.9	W230stop	F159fs#	No SNP	No SNP

*Fold changes in NDM-1 gene coverage to the mean coverage of the entire genome) #fs: frame shift mutation

Impact of porin deletions on the MICs for β-lactam/β-lactamase inhibitor combinations

Strain ID	Resistance Info	FEP	FEP +TAN	FEP +AVI	FEP +VAB	CAZ	CAZ +AVI	MEM	MEM +VAB	ATM	FOX	CHL
C01	NVT1001, parental strain	0.03	0.03	0.03	0.03	0.12	0.12	0.12	0.12	0.059	4	4
C11	Δ*ompK35*	0.06	0.06	0.06	0.06	0.5	0.25	0.12	0.12	0.12	4	4
C12	Δ*ompK36*	0.12	0.06	0.06	0.06	0.12	0.12	0.12	0.12	0.059	8	4
C13	Δ*ompK35* Δ*ompK36*	0.25	0.5	0.25	0.5	0.5	0.5	1	1	0.5	32	4
C14	Δ*ramR*	0.25	0.25	0.25	0.25	1	0.5	0.12	0.12	0.25	32	64
C17	Δ*ompK35* Δ*ompK36* Δ*ramR*	1	1	1	1	2	1	2	2	0.5	128	64
C01-P132	NVT1001(parental strain) / KPC-3	16	0.06	0.06	0.06	128	2	16	0.25	>128	16	2
C11-P132	Δ*ompK35* /KPC-3	64	0.06	0.12	0.12	512	4	32	0.25	>128	64	4
C12-P132	Δ*ompK36* /KPC-3	1024	0.12	0.25	0.25	128	4	32	0.5	>128	64	4
C13-P132	Δ*ompK35* Δ*ompK36* / KPC-3	>1024	1	2	4	512	16	512	64	>128	256	4
C14-P132	Δ*ramR* / KPC-3	32	0.25	0.5	0.25	256	8	32	0.5	>128	128	32
C17-P132	Δ*ompK35* Δ*ompK36* Δ*ramR* / KPC-3	>1024	4	4	16	1024	16	1024	64	>128	512	64

FEP, cefepime; CAZ, ceftazidime; MEM, meropenem; FOX, cefoxitin; CHL, chloramphenicol; ATM, aztreonam. Taniborbactam (TAN) and avibactam (AVI) were fixed at 4 µg/mL, and vaborbactam (VAB) at 8 µg/mL.

Summary of Results

- Selection of *K. pneumoniae* with cefepime-taniborbactam yielded less susceptible mutants.
- Genome sequencing revealed double deletion of OmpK35 and OmpK36 in one mutant, and increased NDM-1 gene copy number in the other mutant.
- Deletions of the porins reduced the activities of cefepime synergistically with KPC-3 in isogenic *K. pneumoniae* strains.
- However, restoration of the cefepime activity by the addition of taniborbactam, were minimally impacted by deletion of the porins.
- Δ*ramR*-mediated increase in efflux had little impact on the activities of cefepime and taniborbactam.
- The deletion of the two major porins reduced the susceptibility to ceftazidime-avibactam and meropenem-vaborbactam to a greater degree.

Conclusions

In *K. pneumoniae*, OmpK35 and OmpK36 were the major porins for penetration of cefepime through the outer membrane into the periplasm. By contrast, taniborbactam does not show a strong dependence on OmpK35 or OmpK36 for periplasmic accumulation.

References

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