β-LACTAMASE INHIBITORS
The Pharmacological Basis of Therapeutics

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INSTITUTE *for* CLINICAL PHARMACODYNAMICS

WARNING! WARNING!!
Potential Conflicts of Interest

- ICPD has ongoing research collaborations involving β-lactam-β-lactamase inhibitor combinations with a number of pharmaceutical companies
  - AiCuris,
  - Cubist Pharmaceuticals/Merck,
  - Fedora/Meiji/Roche,
  - GlaxoSmithKline,
  - The Medicines Company/Rempex, and
  - VenatoRx
- In addition, ICPD is currently conducting studies in support of National Health Service objectives for marketed β-lactamase inhibitors

OUR MISSION

To Boldly Go Where No One Has Gone Before

- What is the PK-PD determinant of β-lactamase inhibitor efficacy in the context of a typical β-lactam exposure?
- What is the impact of β-lactamase gene transcription level on the magnitude of the PK-PD measure associated with β-lactamase inhibitor efficacy?
- Can we identify a translational relationship allowing for the integration of β-lactamase inhibitor exposure-response relationships across isolates?
- Is the translational relationship the same across β-lactams paired with the same β-lactamase inhibitor?

OUR MISSION
To Boldly Go Where No One Has Gone Before

- What is the impact of the partner β-lactam on the PK-PD determinant of β-lactamase inhibitor efficacy in the context of a typical β-lactam exposure?
- Is the PK-PD determinant of β-lactamase inhibitor efficacy the same across β-lactamase inhibitors?
- Is there a basis for the development of a stand-alone β-lactamase inhibitor?
- What is the relationship between β-lactam-β-lactamase inhibitor exposure and resistance amplification?
- How can we utilize pre-clinical model information to support susceptibility breakpoints?

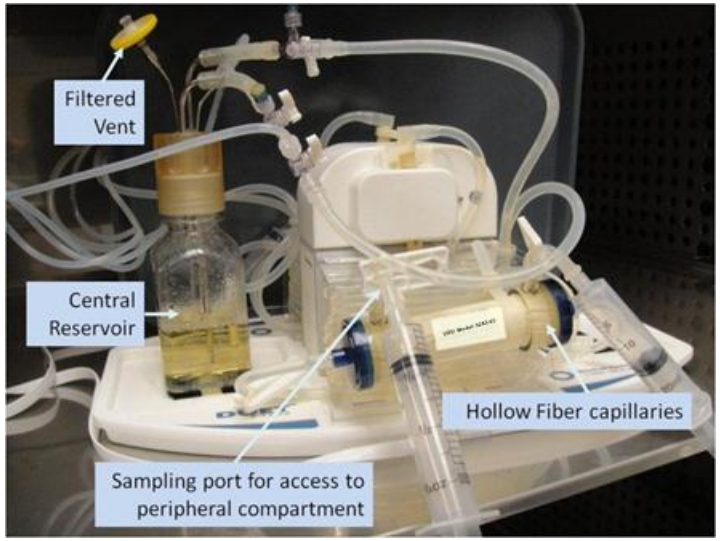
OUR TOOL BOX
One-Compartment Infection Model

- A 24 hour chemostat model was used to measure the impact of a **fractionated β-lactamase inhibitor exposure** on bacterial density in the context of a **fixed β-lactam exposure**
  - Human free-drug concentrations were simulated for each individual agent and measured by LC/MS/MS
  - Starting inoculum was 10^6 CFU/mL
  - Samples collected at 0, 2, 4, 8, 12, and 24 hours and plated on drug-free plates for determination

VanScoy B, Mendes RE, Nicasio AM, Castanheira M, Bulik CC, Okusanya OO, Bhavnani SM, Forrest A, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG. Pharmacokinetics-pharmacodynamics of tazobactam in combination with ceftolozane in an *in vitro* infection model. Antimicrob Agents Chemother 2013;57:2809-2814.

OUR TOOL BOX
Hollow-Fiber Infection Model

- A hollow-fiber model was used identify the **β-lactam-β-lactamase inhibitor** exposure necessary to prevent resistance amplification (Drusano/Louie)
  - Human free-drug concentrations were simulated for each individual agent and measured by LC/MS/MS
  - Starting inoculum was 10^8 CFU/mL
  - Samples were collected daily over 10-14 days and plated on drug-free and –containing plates for CFU determination
  - Samples were collected over the first 48 hours for drug assay



VanScoy B, Mendes RE, Castanherira M, McCauley J, Bhavnani SM, Forrest A, Okusanya OO, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG. Relationship between ceftolozane/tazobactam exposure and drug-resistance amplification in a hollow-fiber infection model. *Antimicrob Agents Chemother* 2013;57:4134-4138.

What is the PK-PD determinant of β-lactamase inhibitor efficacy in the context of a typical β-lactam exposure?

DOSE FRACTIONATION STUDIES
Strains and Susceptibility Testing

- Three isogenic CTX-M-15-elaborating *E. coli* were utilized in these studies
  - Genetically engineered $bla_{CTX\text{-}M\text{-}15}$-carrying vectors containing varying upstream promoter regions that provided different levels of mRNA transcription were created
  - Recombinant vectors were then transformed into a wild-type susceptible *E. coli* strain

Strain	Genetic Construct	Hydrolytic Activity[1]	qRT-PCR	MIC (mg/L)			
				CXA-101	CXA-101 Tazo (4 mg/L)	Piperacillin	Piperacillin Tazo (4 mg/L)
E. coli 120-3863A	Wild-Type	-3	ND	0.5	0.5	2	2
E. coli JMI 10768	Low	36	1	4	0.25	64	2
E. coli JMI 11103	Medium	120	8.3	16	0.25	256	2
E. coli JMI 10770	High	580	43.9	64	0.25	512	2

1: ceftolozane (mg) hydrolyzed per min per mg of protein

VanScoy B, Mendes RE, Nicasio AM, Castanheira M, Bulik CC, Okusanya OO, Bhavnani SM, Forrest A, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG. Pharmacokinetics-pharmacodynamics of tazobactam in combination with ceftolozane in an *in vitro* infection model. *Antimicrob Agents Chemother* 2013;57:2809-2814.

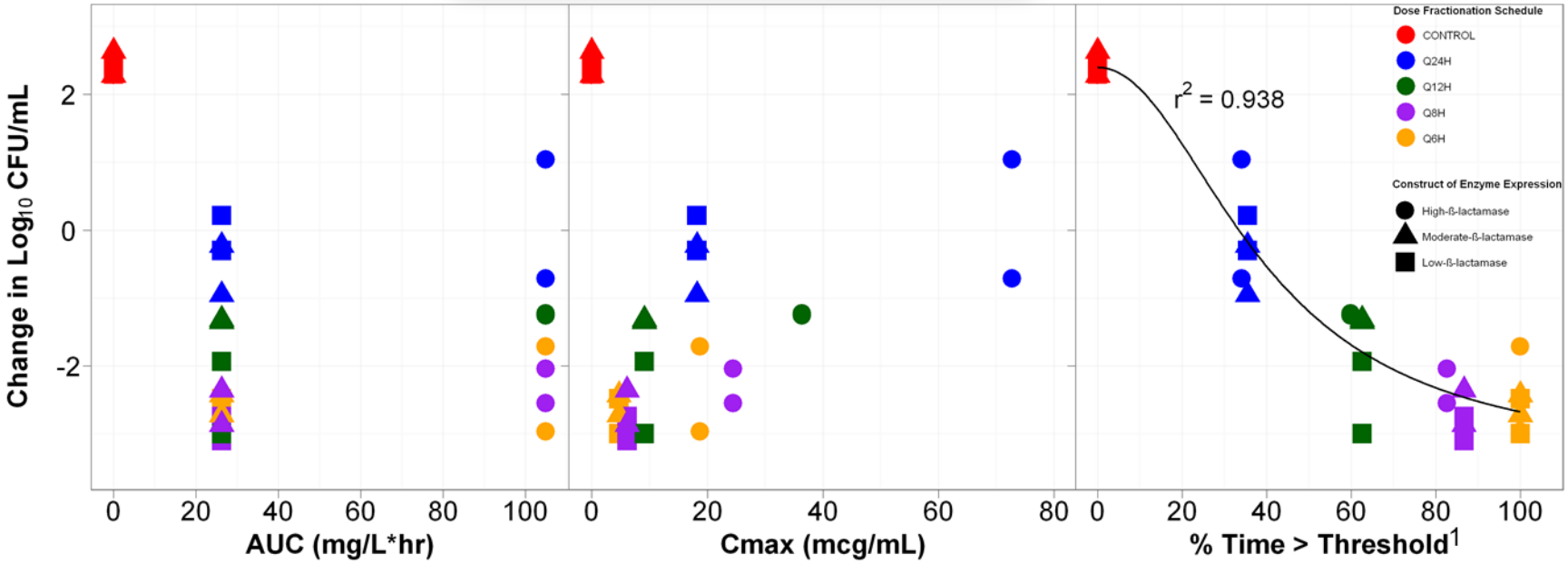
DOSE FRACTIONATION STUDIES
PK-PD Modeling

- Data from the efficacy studies were modeled using a Hill-type model and non-linear least squares regression
  - The data were weighted using the inverse of the estimated measurement variance
  - The relationship between $\log_{10}$ CFU at 24 hours and C_{max}, AUC_{0-24}, and % Time>threshold was evaluated
- The % Time>threshold was identified through an iterative process
  - Candidate threshold concentrations of 0.01, 0.05, 0.1, 0.25, 0.5, 1, and 2 mg/L were evaluated
  - Threshold value discrimination was based upon resolution along the exposure axis and r^2 optimization

VanScoy B, Mendes RE, Nicasio AM, Castanheira M, Bulik CC, Okusanya OO, Bhavnani SM, Forrest A, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG. Pharmacokinetics-pharmacodynamics of tazobactam in combination with ceftolozane in an *in vitro* infection model. *Antimicrob Agents Chemother* 2013;57:2809-2814.

DOSE FRACTIONATION STUDIES
Tazobactam Exposure-Response In Vitro

Ceftolozane-Tazobactam



1: The threshold tazobactam concentration for the low- and moderate-β-lactamase genetic constructs was 0.05 mg/L and was 0.25 mg/L for the high-β-lactamase genetic construct

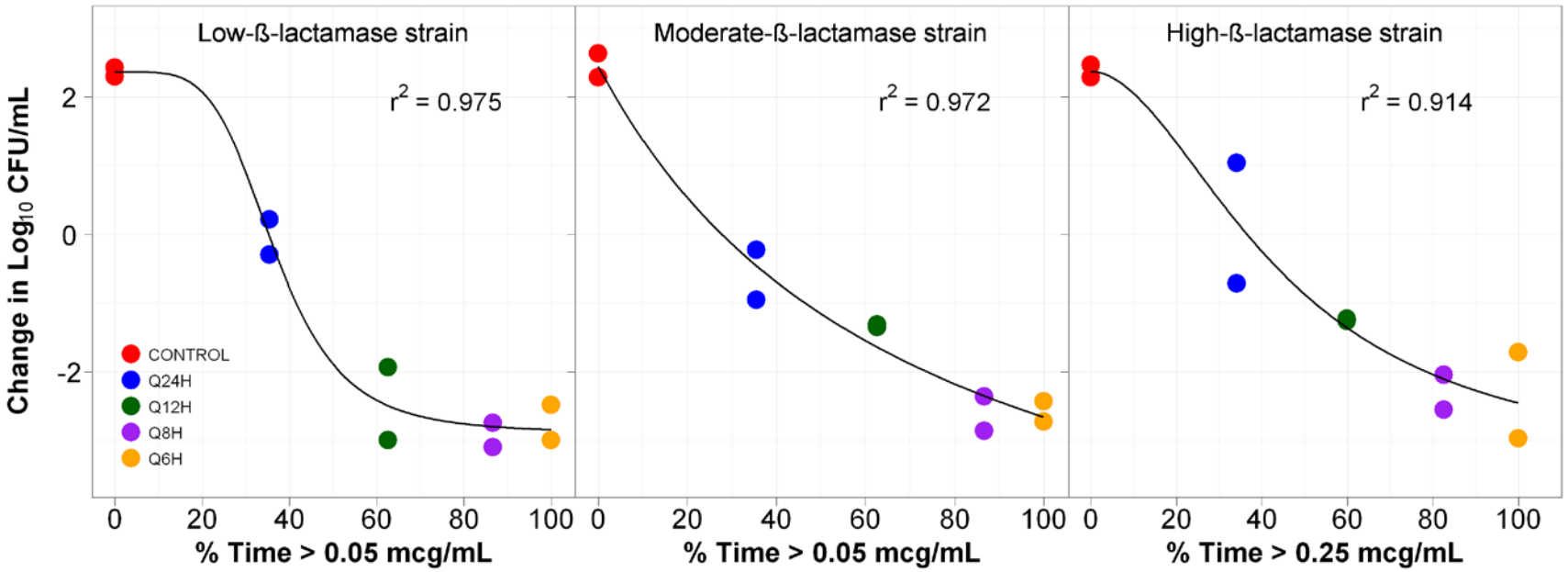
For tazobactam in combination with ceftolozane, % Time > Threshold is the PK-PD determinant of β-lactamase inhibitor efficacy

VanScoy B, Mendes RE, Nicasio AM, Castanheira M, Bulik CC, Okusanya OO, Bhavnani SM, Forrest A, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG. Pharmacokinetics-pharmacodynamics of tazobactam in combination with ceftolozane in an *in vitro* infection model. *Antimicrob Agents Chemother* 2013;57:2809-2814.

What is the impact of β-lactamase gene transcription level on the magnitude of the PK-PD measure associated with β-lactamase inhibitor efficacy?

GENE TRANSCRIPTION IMPACT
Tazobactam Exposure-Response In Vitro

Ceftolozane-Tazobactam



As gene transcription level increases, so too does the β-lactamase inhibitor target threshold

VanScoy B, Mendes RE, Nicasio AM, Castanheira M, Bulik CC, Okusanya OO, Bhavnani SM, Forrest A, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG. Pharmacokinetics-pharmacodynamics of tazobactam in combination with ceftolozane in an *in vitro* infection model. *Antimicrob. Agents Chemother* 2013;57:2809-2814.

Can we identify a translational relationship allowing for the integration of β-lactamase inhibitor exposure-response relationships across isolates?

DOSE RANGE STUDIES
Strains and Susceptibility Testing

Resistance mechanisms	*E. coli* clinical isolate and ceftolozane/tazobactam MIC [µg/mL][a]			
	4643 [0.5]	1801 [0.5]	21711 [2]	13319 [4]
β-lactamase gene[b]	$bla_{CTX-M-15}$	$bla_{CTX-M-15}$	$bla_{CTX-M-15}$	$bla_{CTX-M-15}$
	$bla_{OXA-1/30}$		$bla_{OXA-1/30}$	bla_{TEM-1}
			bla_{TEM-1}	
Hydrolysis assay[c]	0.83	0.16	3.50	2.43
Expression results[d]				
CTX-M-15	1 (0.6-1.7)	5.6 (4.9-6.3)	14.5 (10.5-20.0)	2.0 (1.3-3.0)
AmpC	2.1 (1.7-2.6)	4.2 (2.8-6.4)	2.4 (2.1-2.8)	2.0 (1.6-2.5)
AcrAB-TolC	1.3 (1.1-1.5)	10.7 (8.3-13.8)	1.6 (1.3-2.0)	3.5 (2.7-4.4)
OmpC	2,316 (1,919-2,795)	3,123.5 (2,455-3,973)	3,137 (2,805-3,508)	0.51 (0.36-0.73)
OmpF	1.0 (0.7-1.4)	0.25 (0.19-0.33)	0.88 (0.87-0.89)	0.44 (0.34-0.56)

[a] Represent a modal MIC value from triplicate results obtained using frozen-form panels manufactured according to the CLSI (M07-A9, 2012) specifications.

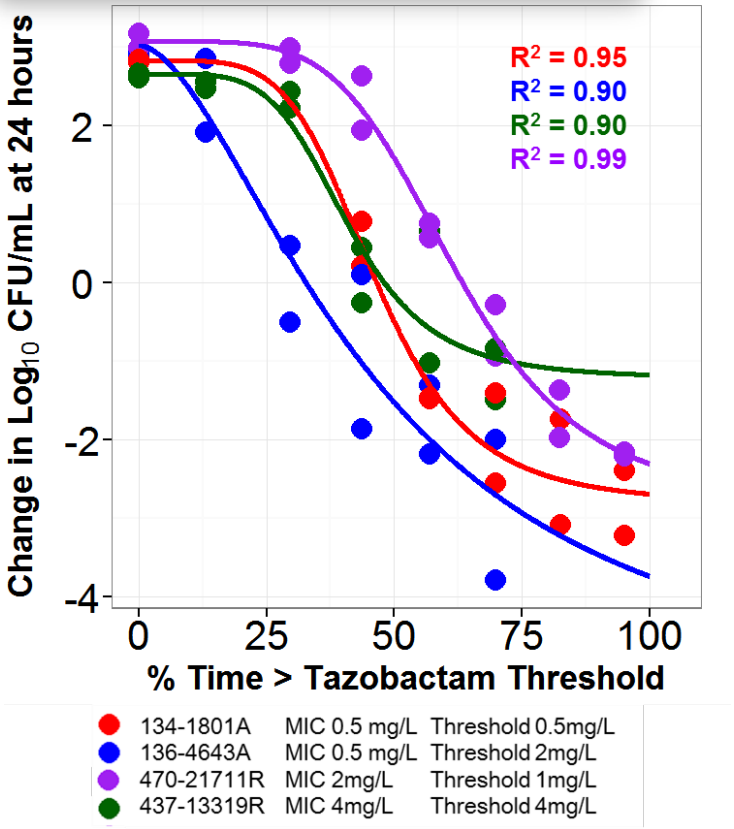
[b] β-lactamase gene content determined by PCR and confirmed by sequencing analysis.

[c] Hydrolytic activity rates expressed as substrate (nitrocefin) hydrolyzed (Δ Absorbance) per minute per mg of protein.

[d] Quantification of transcriptional levels for $bla_{CTX-M-15}$ (relative to *rpsL* endogenous reference). Results obtained were compared against that obtained from the strain with the lowest expression levels (i.e. *E. coli* 4643). The *ampC*, *acrA*, *ompC* and *ompF* transcriptional levels were compared to those obtained from a clinically relevant wild-type ST131 *E. coli* isolate (ceftolozane/tazobactam MIC, 0.25 µg/mL) (VanScoy et al., 2013). Values between parentheses represent the respective relative quantification ± the standard deviation value. Shaded areas represent expression values that may contribute for the decreased susceptibility to ceftolozane/tazobactam.

DOSE-RANGE STUDIES
Tazobactam Exposure-Response In Vitro

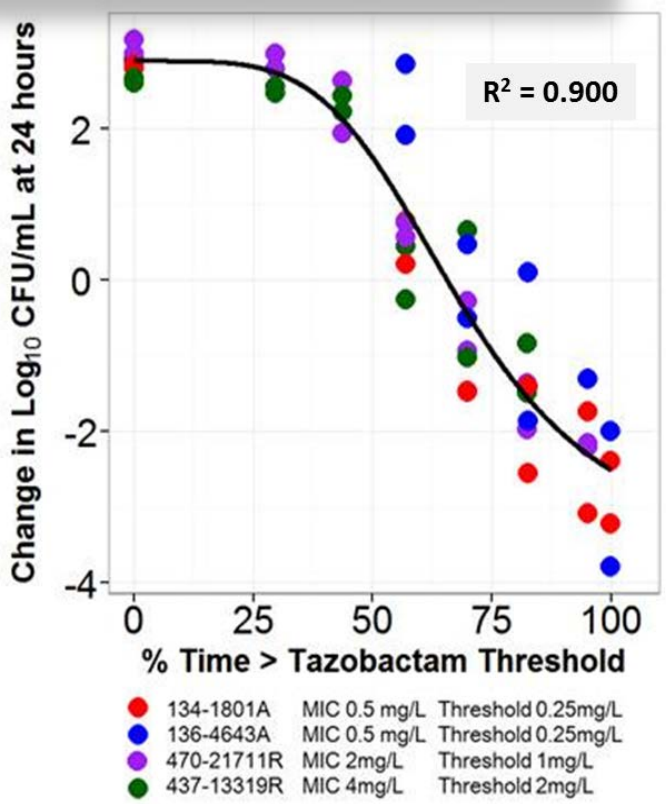
Ceftolozane-Tazobactam



VanScoy B, Mendes RE, McCauley J, Bhavnani SM, Bulik CC, Okusanya OO, Forrest A, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG. Pharmacological basis of β-lactamase therapeutics: tazobactam in combination with ceftolozane. *Antimicrob Agents Chemother* 2013;57:5924-5930.

TRANSLATIONAL RELATIONSHIP
Tazobactam Exposure-Response In Vitro

Ceftolozane-Tazobactam



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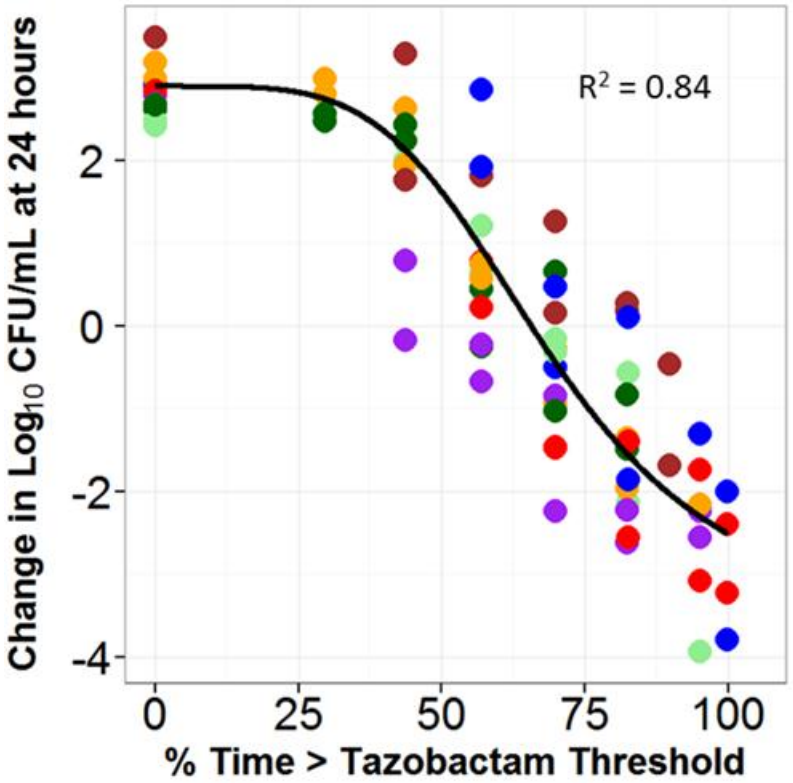


Escherichia coli

1801A	MIC 0.5 mg/L	Threshold 0.25mg/L
4643E	MIC 0.5 mg/L	Threshold 0.25mg/L
21711R	MIC 2mg/L	Threshold 1mg/L
13319R	MIC 4mg/L	Threshold 2mg/L

Klebsiella pneumoniae

604C	MIC 1 mg/L	Threshold 0.5mg/L
21904E	MIC 2 mg/L	Threshold 1mg/L
4812E	MIC 4mg/L	Threshold 2mg/L

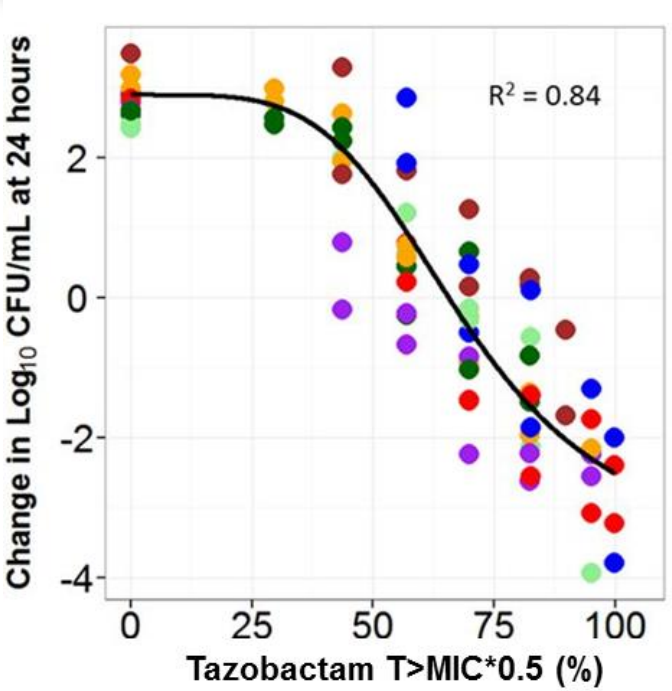
For ceftolozane-tazobactam, a translational relationship was identified and allowed for the integration of β-lactamase inhibitor exposure-response relationships across isolates

VanScoy B, Mendes RE, McCauley J, Bhavnani SM, Bulik CC, Okusanya OO, Forrest A, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG. Pharmacological basis of β-lactamase therapeutics: tazobactam in combination with ceftolozane. Antimicrob Agents Chemother 2013;57:5924-5930.

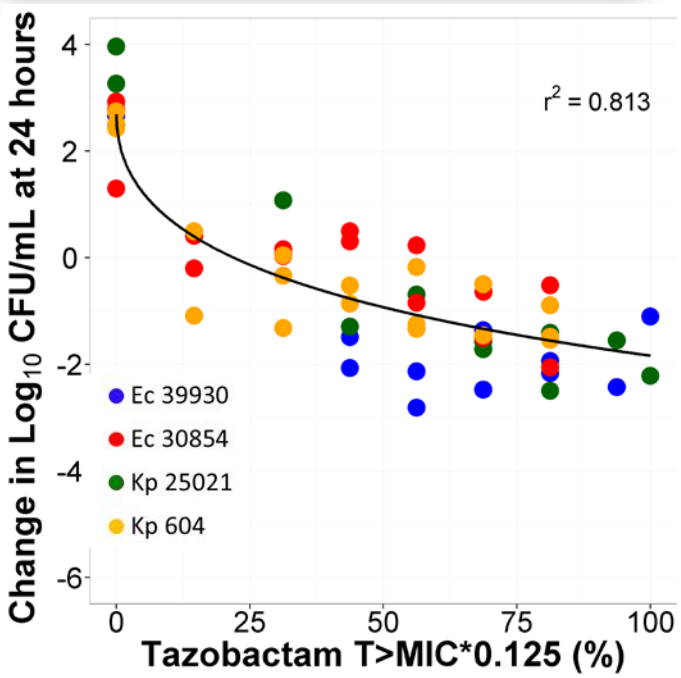
Is the translational relationship the same across β-lactams paired with the same β-lactamase inhibitor?

TRANSLATIONAL RELATIONSHIP
Tazobactam Exposure-Response In Vitro

Ceftolozane-Tazobactam



Cefepime-Tazobactam



The translational relationship is not the same across β-lactam partners

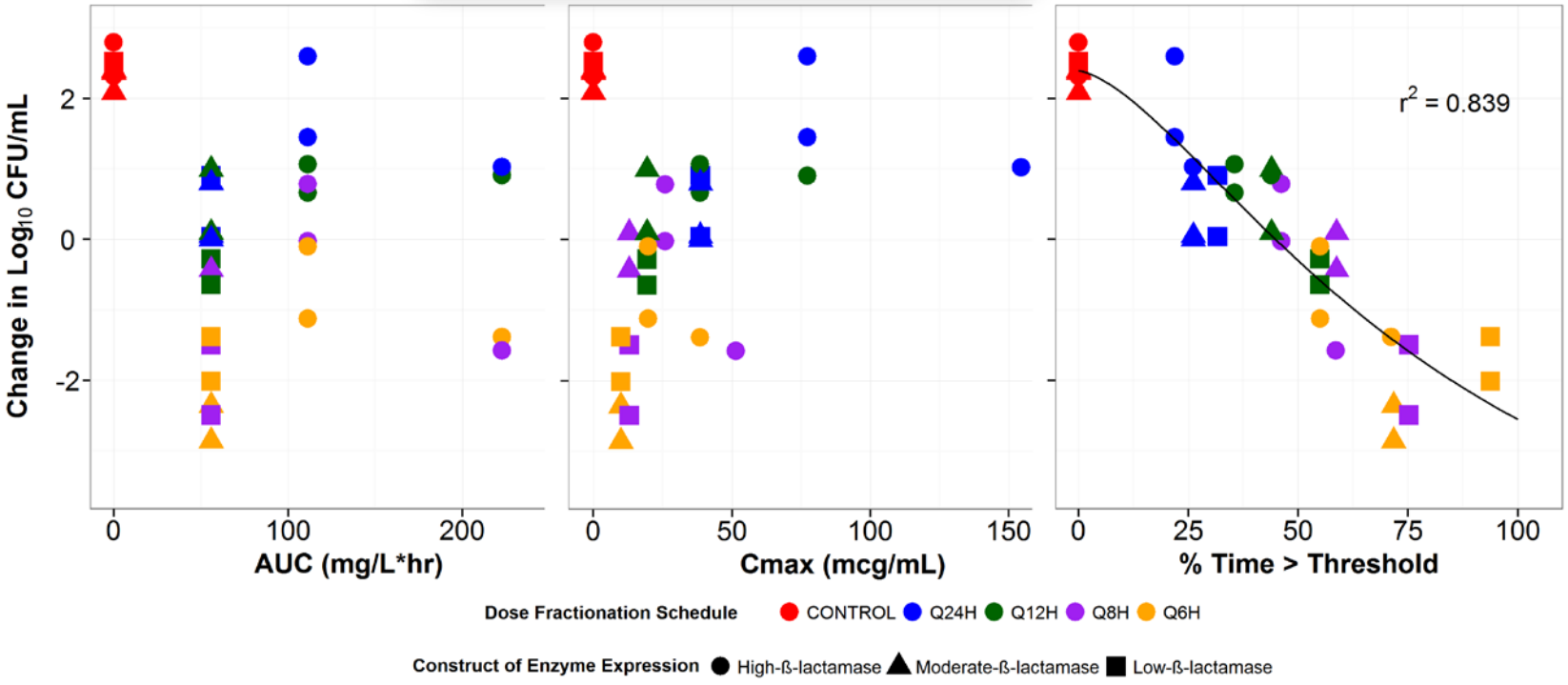
VanScoy BD, Mendes RE, McCauley J, Bhavnani SM, Bulik CC, Okusanya OO, Forrest A, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG. Pharmacological basis of β-lactamase therapeutics: tazobactam in combination with ceftolozane. Antimicrob Agents Chemother 2013;57:5924-5930.

VanScoy BD, Tenero D, Turner S, Livermore DM, McCauley J, Conde H, Mendes R, Bhavnani SM, Rubino CR, Ambrose PG. Pharmacokinetics-pharmacodynamics of tazobactam in combination with cefepime in an *in vitro* infection model. Poster A-499, 2015 ICAAC.

What is the impact of the partner β-lactam on the PK-PD determinant of β-lactamase inhibitor efficacy in the context of a typical β-lactam exposure?

DOSE FRACTIONATION STUDIES
Tazobactam Exposure-Response In Vitro

Piperacillin-Tazobactam



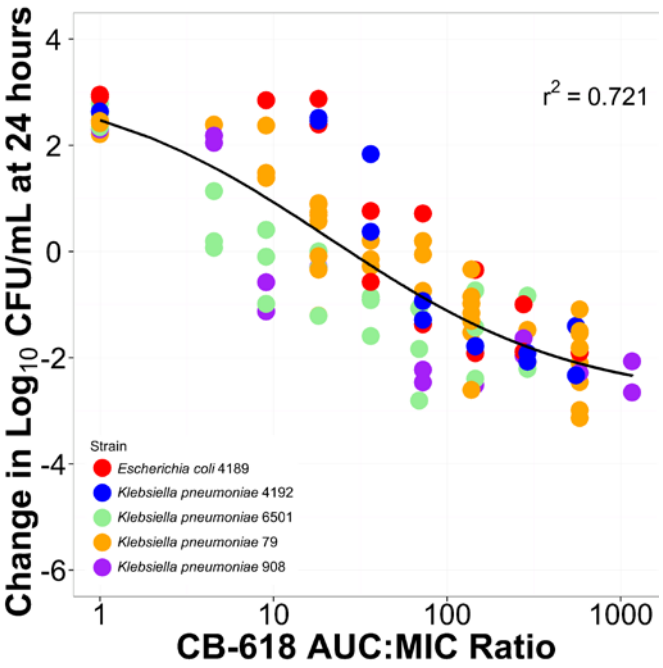
1: The threshold tazobactam concentration for the low-, moderate-, and high-β-lactamase genetic constructs were 0.25, 0.5, and 2 mg/L, respectively.

PK-PD determinant of β-lactamase inhibitor efficacy does not change with the β-lactam partner

Is the PK-PD determinant of β-lactamase inhibitor efficacy the same across β-lactamase inhibitors?

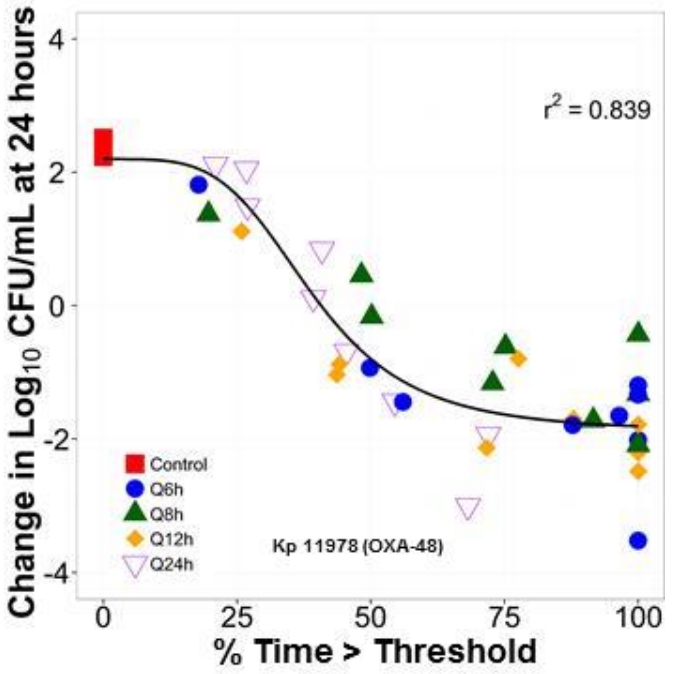
PK-PD EFFICACY DETERMINANTS
Various β-Lactamase Inhibitors with Meropenem

CB-618
Meropenem



VanScoy BD, Rubino CM, McCauley J, Conde H, Bhavnani SM, Friedrich LV. Alexander DC, Ambrose PG. Pharmacokinetics-pharmacodynamics of CB-618, a novel β-lactamase inhibitor, in combination with meropenem in an *in vitro* infection model. Poster A-044, 2015 ICAAC.

Diazabicyclooctane
Meropenem



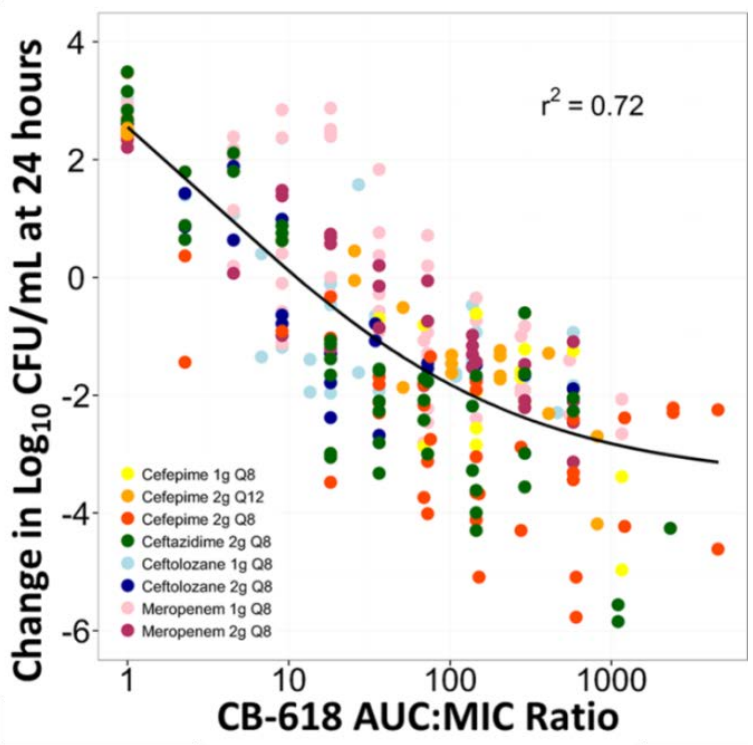
No, the PK-PD determinant of β-lactamase inhibitor efficacy is not the same across β-lactamase inhibitors

Is there a basis for the development of a stand-alone β-lactamase inhibitor?

BASIS FOR A STAND ALONE INHIBITOR?

Potential to Rescue Multiple Agents and Regimens

Isolate	Enzyme	MER	TOL	PIM	CAZ
K. pneumoniae 79	KPC-3	●	●	●	
K. pneumoniae 908	KPC-2	●	●	●	
K. pneumoniae 6501	KPC-3			●	●
K. pneumoniae 9380	KPC-2		●		
K. pneumoniae 4192	OXA-48	●			
E. coli 4189	OXA-48	●			
E. coli 4643	CTX-M-15			●	●
E. cloacae 4182	AmpC				●

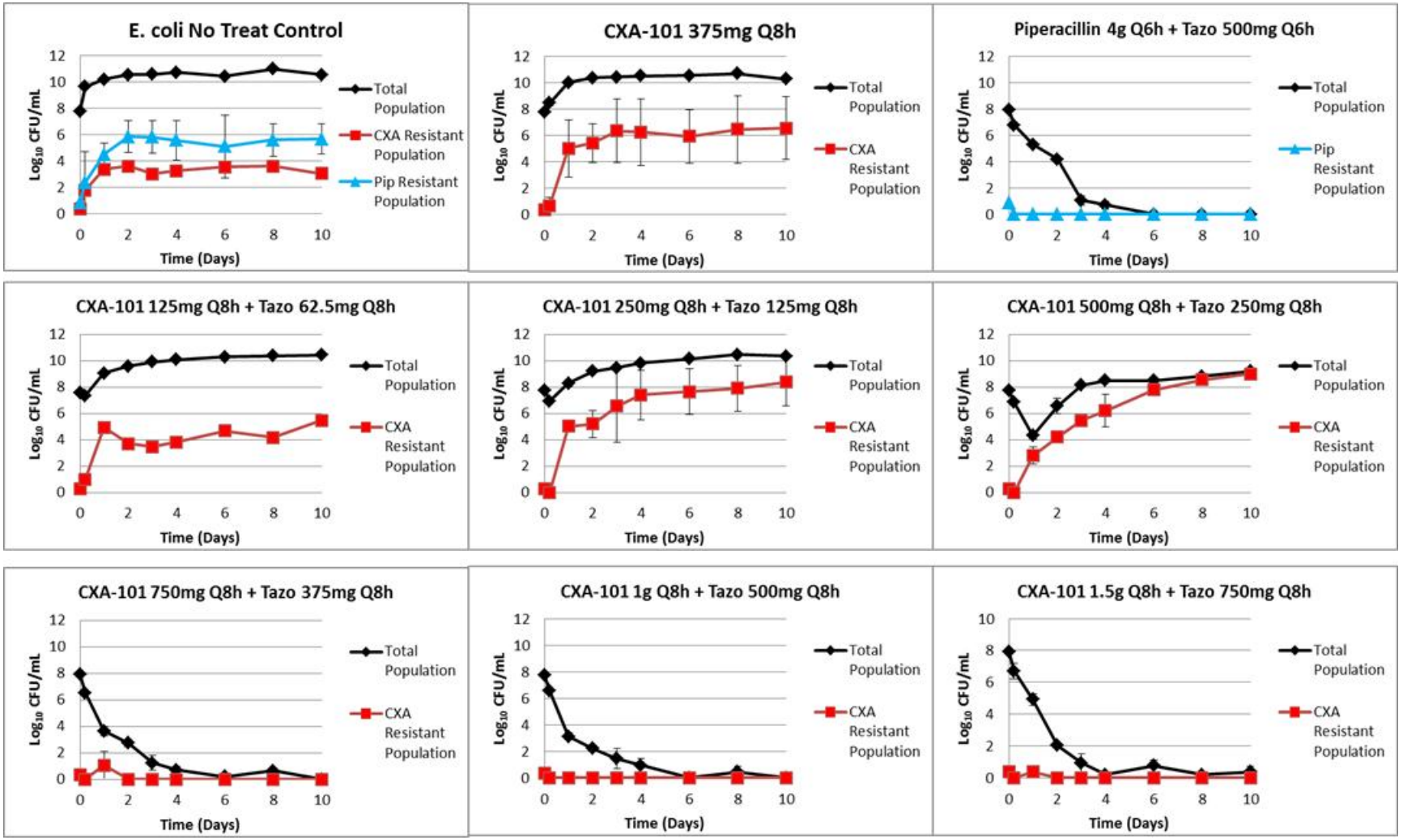


From a PK-PD perspective, it is possible to identify one β-lactamase inhibitor exposure to rescue multiple β-lactams and dosing regimens

VanScoy BD, Rubino CM, McCauley J, Conde H, Bhavnani SM, Friedrich LV. Alexander DC, Ambrose PG. Pharmacokinetics-pharmacodynamics of CB-618 in combination with multiple β-lactam agents. Poster A-501, 2015 ICAAC.

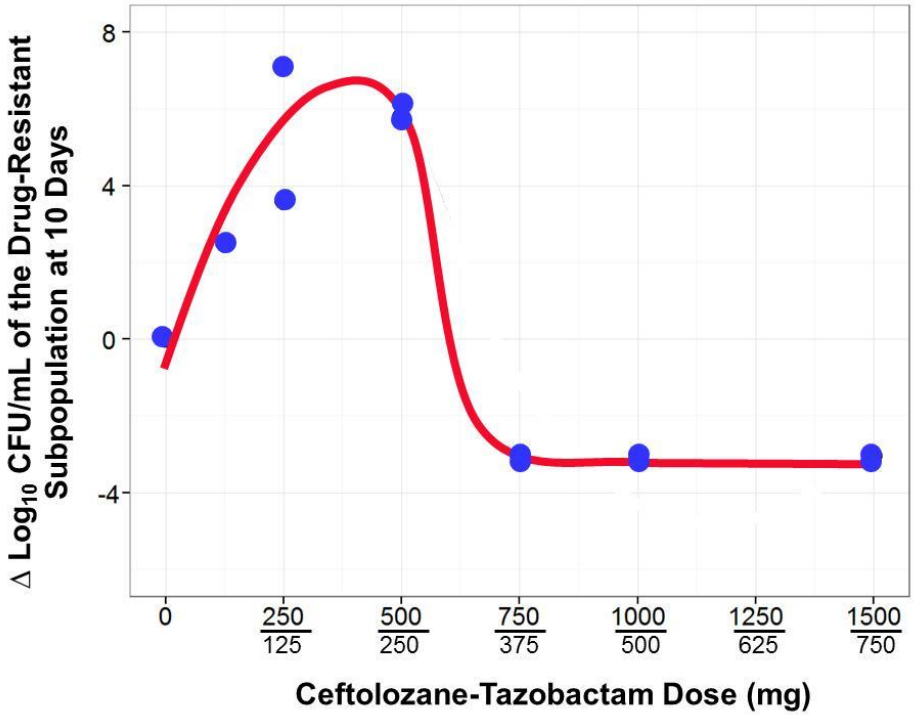
What is the relationship between β-lactam-β-lactamase inhibitor exposure and resistance amplification?

CEFTOLOZANE-TAZOBACTAM
On-Therapy Resistance Amplification



Note: Averaged data with error bars representing the range of data over two separate studies.

CEFTOLOZANE-TAZOBACTAM
On-Therapy Resistance Amplification



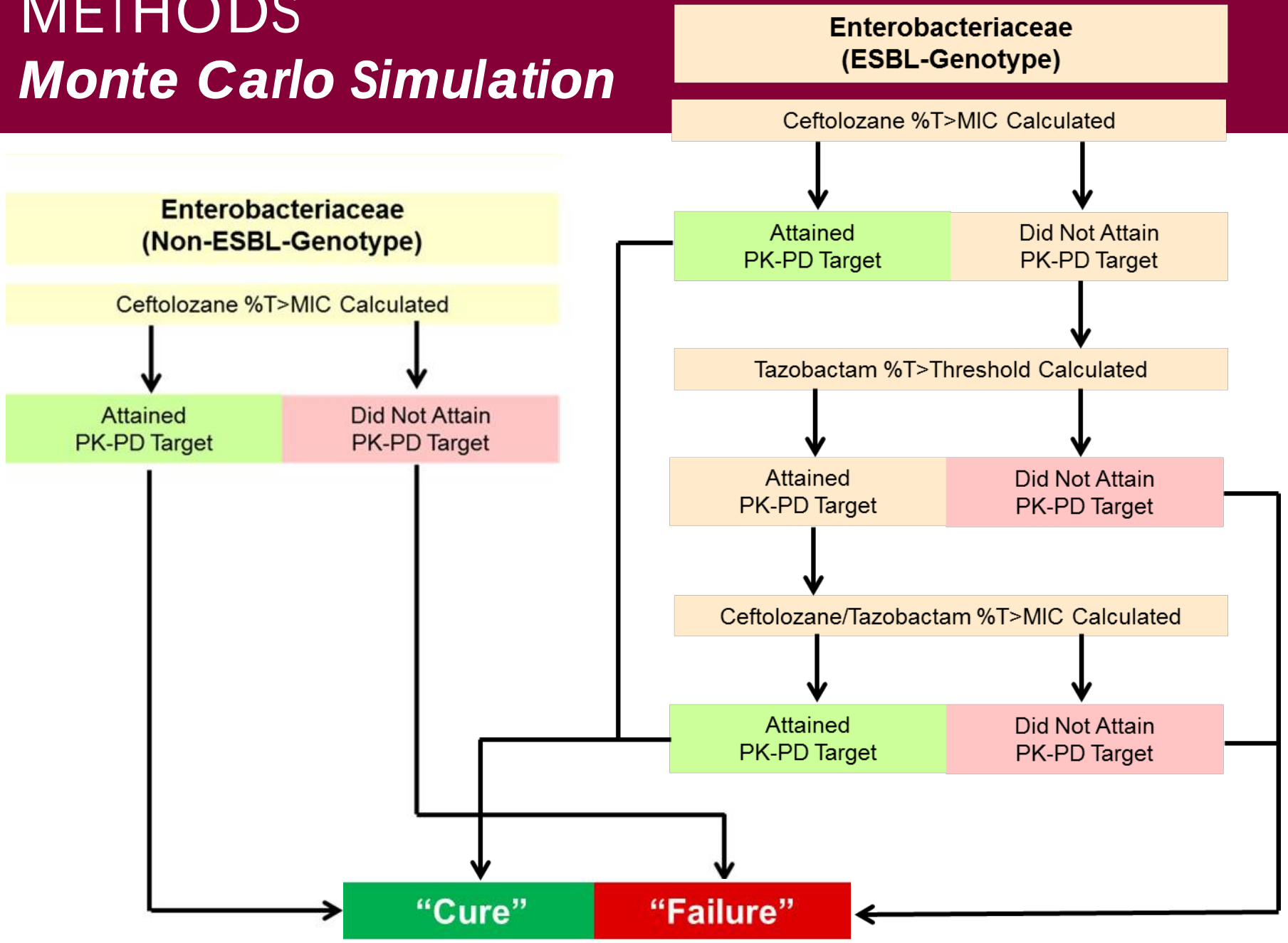
Strain	*E. Coli* JMI 11103	
Enzyme		CTX-M-15
MIC (mg/L)	TOL TOL/TAZ	16 0.25
Hydrolytic Activity		120
qRT-PCR		8.3

The relationship between ceftolozane-tazobactam exposure and resistance amplification is that of an inverted U

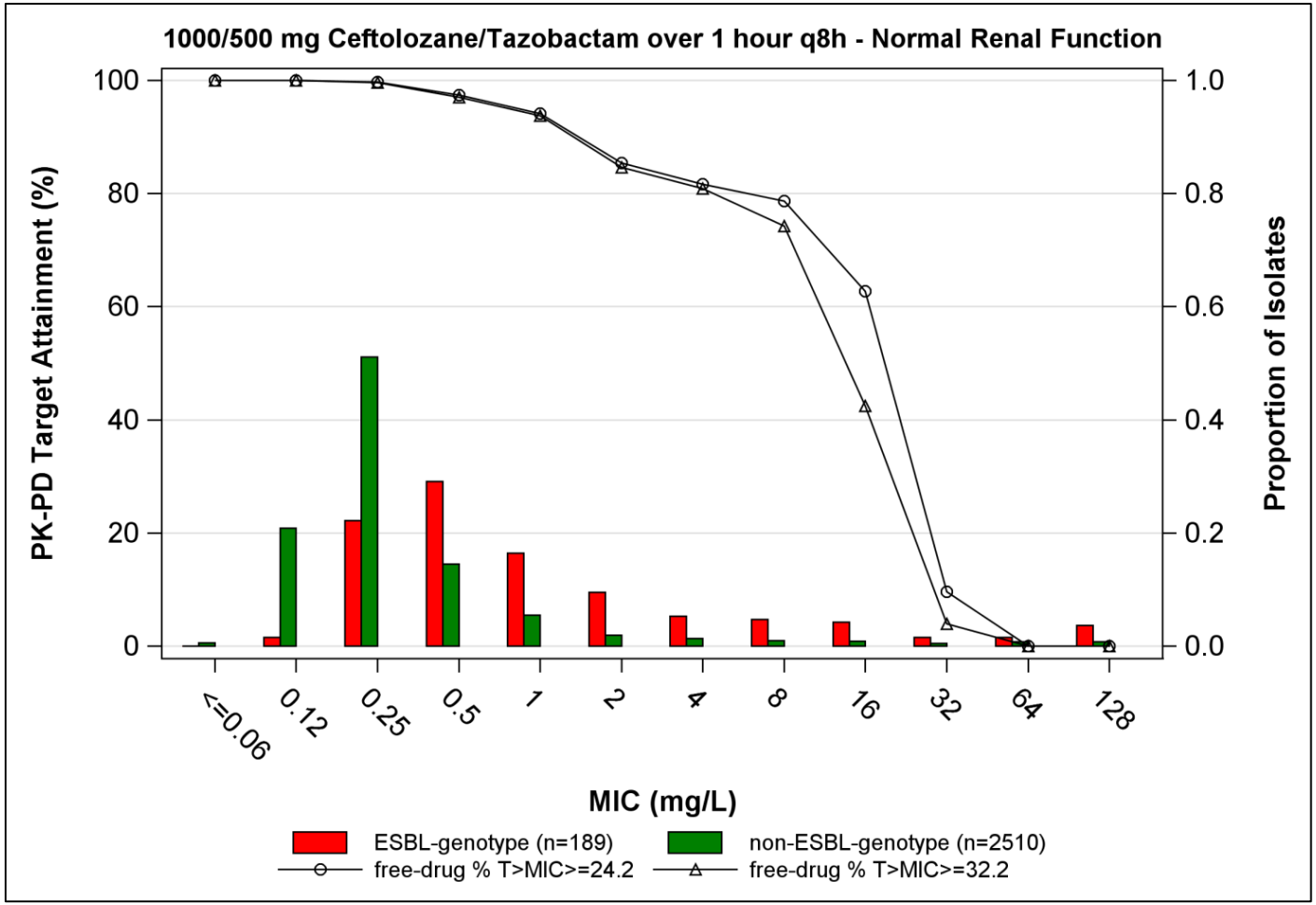
VanScoy B, Mendes RE, Castanherira M, McCauley J, Bhavnani SM, Forrest A, Okusanya OO, Jones RN, Friedrich LV, Steenbergen JN, Ambrose PG. Relationship between ceftolozane/tazobactam exposure and drug-resistance amplification in a hollow-fiber infection model. Antimicrob Agents Chemother 2013;57:4134-4138.

How can we utilize pre-clinical model information to support susceptibility breakpoints?

METHODS
Monte Carlo Simulation



CEFTOLOZANE-TAZOBACTAM
Probability of Target Attainment



Rubino CM, Bhavnani SM, Steenbergen JN, Krishna G. Ambrose PG. Pharmacokinetic-pharmacodynamic target attainment analysis supporting the selection of *in vitro* susceptibility test interpretive criteria for ceftolozane/tazobactam against Enterobacteriaceae. Poster A-1347. ICAAC 2014.

SUMMARY
Dose Selection of β-Lactamase Inhibitors

- Know the PK-PD determinate of the β-lactamase inhibitor
  - They are not all the same!
- Know the β-lactamase inhibitor exposure magnitude needed for efficacy in combo with the β-lactam dose regimen you will study clinically
  - Look for unifying translational relationships across isolates to increase certainty around dose regimen decisions
- Dose justification and breakpoint evaluations require consideration of both β-lactam and β-lactamase inhibitor exposures
- Pressure test clinical regimens in hollow-fiber infection models prior to clinical trials

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