Establishment of Murine Thigh Infection Models with Clinical Isolates of MDR *Klebsiella pneumoniae* for Pharmacology Studies

Pharmacology Discovery Services

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Abstract

Background: This study aimed to establish mouse thigh infection models with characterized MDR *K. pneumoniae* clinical isolates for efficacy evaluations of antibacterial drug candidates. The models use MDR organisms from the FDA-CDC AR Isolate Bank including ESBL-, KPC-, and NDM-producing organisms. The antibiotic susceptibility and genome sequences are available from the AR-Bank. This presentation describes protocols for the murine models and efficacy of tigecycline (TGC) or colistin (CST) as reference drugs.

Approach and Methods: Thigh infection studies were conducted with neutropenic ICR mice (Andes and Craig 2002). The models were first optimized by performing titrations of the bacterial inoculum. Efficacy analysis of reference antibiotics, TGC and CST, were evaluated for model validation and benchmarking. TGC was dosed from 12.5 to 200 mg/kg/24 h. CST was dosed from 2 to 80 mg/kg for AR-BANK#0138 which is TGC-intermediate. Dose administration, BID q12h, started 2 h after infection for each reference drug. Testing with QID q6h dosing was conducted with selected doses. Animal groups (N=5) were sacrificed 2 h after infection for initial bacterial counts at baseline or 26 h for final counts in thigh tissue. The significance of antibacterial effects was determined with ANOVA.

Results: All strains grew well in thigh tissue, resulting in a ≥2-$\log_{10}$ increase in counts with an inoculum of 10^5 CFU with most strains. Treatments with reference drugs, TGC and CST, resulted in significant dose-responsive growth inhibition of all five clinical isolates (Table). A 1-log killing effect was observed with three of the isolates.

Conclusions: This collection of characterized MDR *K. pneumoniae* isolates and infection protocols will enable researchers to test the therapeutic value of their drug candidates *in vivo* against pathogens with priority antibiotic resistance mechanisms.

Methods

Bacterial strains: All strains are from the American Type Culture Collection (ATCC) or the FDA-CDC Antimicrobial Resistance Isolate Bank.

Animal preparation: Specific pathogen free female ICR mice weighing 22 ± 2 g were used. Cyclophosphamide (CP) was administered IP at 150 mg/kg (Day –4) and 100 mg/kg (Day –1) to induce neutropenia, < 100 neutrophils per µL.

Inoculum preparation: A 0.2 mL aliquot of a single-use glycerol stock (at -80 °C) was used to seed 20 mL brain heart infusion (BHI) and then incubated at 35-37 °C with shaking (250 rpm) for 8 h. Cells in the 20 mL culture were suspended in 10 mL cold PBS. OD measurements were used to guide dilution to the target inoculum count. The PBS suspension was stored on ice for no more than one h.

Challenge: On Day 0, animals were infected with pathogen suspension (table below) by intramuscular injection into the right thigh, 0.1 mL. A titration of the pathogen suspension was first conducted to determine the optimal titer that results in approximately 10^6 CFU/g at baseline, the time of test article administration (Bulitta 2019).

Antimicrobial Treatment: TYGACIL® (tigecycline, Wyeth Lederle SRL, Italy) and Colistin sulfate (C4461 Sigma USA) were formulated in 0.9% NaCl and administered, twice (BID, q12h intervals) or four times (QID q6h intervals) by subcutaneous (SC) injection, 10 mL/kg, starting at 2 h after infection. The dosing was selected from published reports (Van van Ogtrop 2000, Landersdorfer 2018).

Tissue harvest. Animals were sacrificed with CO_2 asphyxiation at the scheduled time points for tissue harvest, at 2 h (baseline) or 26 h after infection. Thigh muscle tissue was aseptically harvested, weighed, and homogenized in 3 mL sterile PBS (pH 7.4) with a Polytron homogenizer. Bacterial density in the tissue homogenates was determined by performing 10-fold serial dilutions and plating 0.1 mL of each to NA plates. Colonies were counted after 18 – 24 h incubation. The colony forming units per g tissue (CFU/g) were calculated.

Data analysis. Mean and SEM values were calculated. The difference, Δ, in bacterial density between treatment group and the baseline group (2 h initial counts) group was calculated and the significance was assessed (ANOVA, Prism) with a criteria of $p < 0.05$ The doses that result in a net static effect (bacteriostasis) and a 1-$\log_{10}$ reduction in counts relative to baseline were estimated with nonlinear regression using GraphPad Prism.

Bacterial Strains

Sequence type and MMR

Strain ID	Genome Accession	Sequence Type	Molecular Mechanism of β-lactam Resistance*	
			β-lactam	Truncated porin
ATCC 43816	GCF_000742755.1	493		
AR-BANK#0087	SAMN04014928	16	**SHV-12**	OmpK35
AR-BANK#0098	SAMN04014939	258	**KPC-2**, OXA-9, TEM-1A	OmpK35
AR-BANK#0097	SAMN04014938	2167~	**KPC-3**, OXA-9, SHV-11, TEM-1A	
AR-BANK#0041	SAMN04014882	37	**NDM-1**, CMY-4, CTX-M-15, OXA-10, SHV-11	
AR-BANK#0135	SAMN04014976	101	**VIM-1**, OXA-9, SHV-12, TEM-1A	OmpK35
AR-BANK#0160	SAMN04015001	36	**OXA-48**, SHV-11	
AR-BANK#0138	SAMN04014979	147	**NDM-7**, CTX-M-15, SHV-11, TEM-1B	

*FDA-CDC Antimicrobial Resistance Isolate Bank, genomic analysis.
Sequence types were determined using whole genome sequence and the PubMLST database.

Antibiotic Susceptibility

Strain ID	Notable MMR	TZP	ATM	CAZ	CZA	MEM	AMK	LVX	CST	TGC
ATCC 43816		S	S	S	S	S	S	S	S	S
AR-BANK#0087	SHV-12	R	R	R	S	S	S	R	R	S
AR-BANK#0098	KPC-2	R	R	R	S	R	R	R	S	S
AR-BANK#0097	KPC-3	R	R	R	R	R	R	R	R	S
AR-BANK#0041	NDM-1	R	R	R	R	R	R	R	S	S
AR-BANK#0135	VIM-1	R	R	R	R	R	S	R	S	S
AR-BANK#0160	OXA-48	R	S	S	S	R	S	S	S	S
AR-BANK#0138	NDM-7	R	R	R	R	R	R	R	S	I

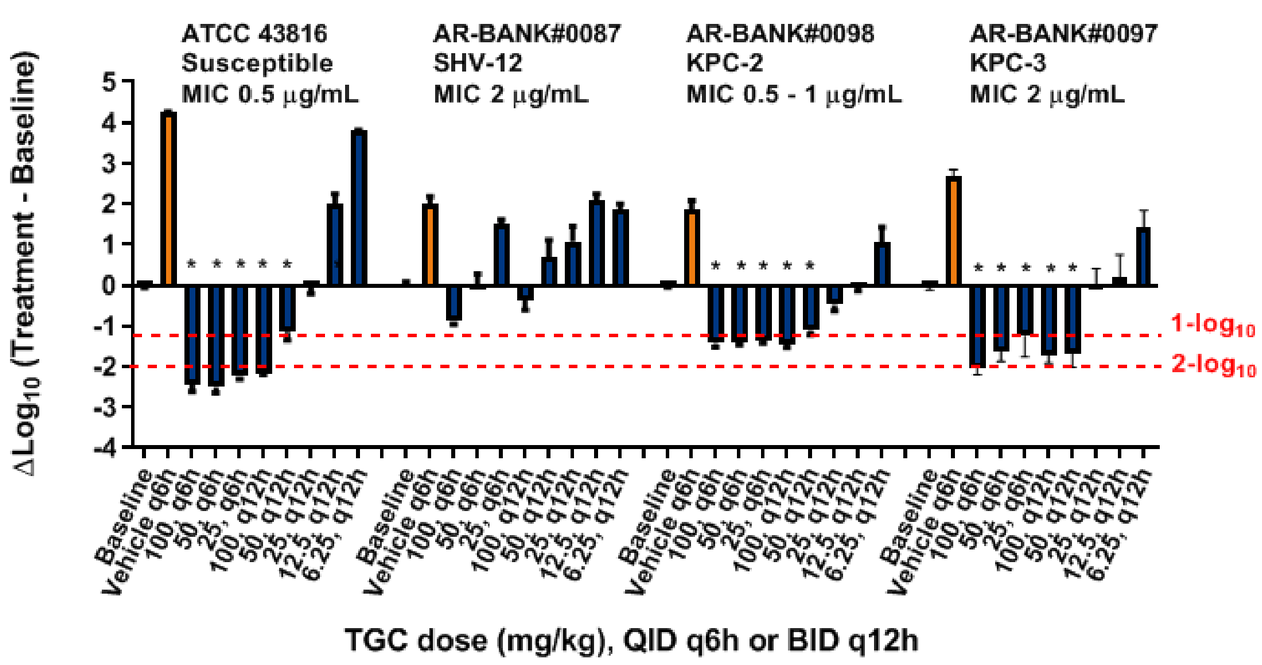
Abbreviations: Piperacillin Tazobactam TZP, Aztreonam ATM, Ceftazidime CAZ, Ceftazidime avibactam, CZA, meropenem MEM, amikacin AMK, levofloxacin LVX, colistin CST, and tigecycline TGC
Susceptibility to antibiotics was conducted following CLSI guidelines and interpretive criteria from CLSI, EUCAST and FDA.

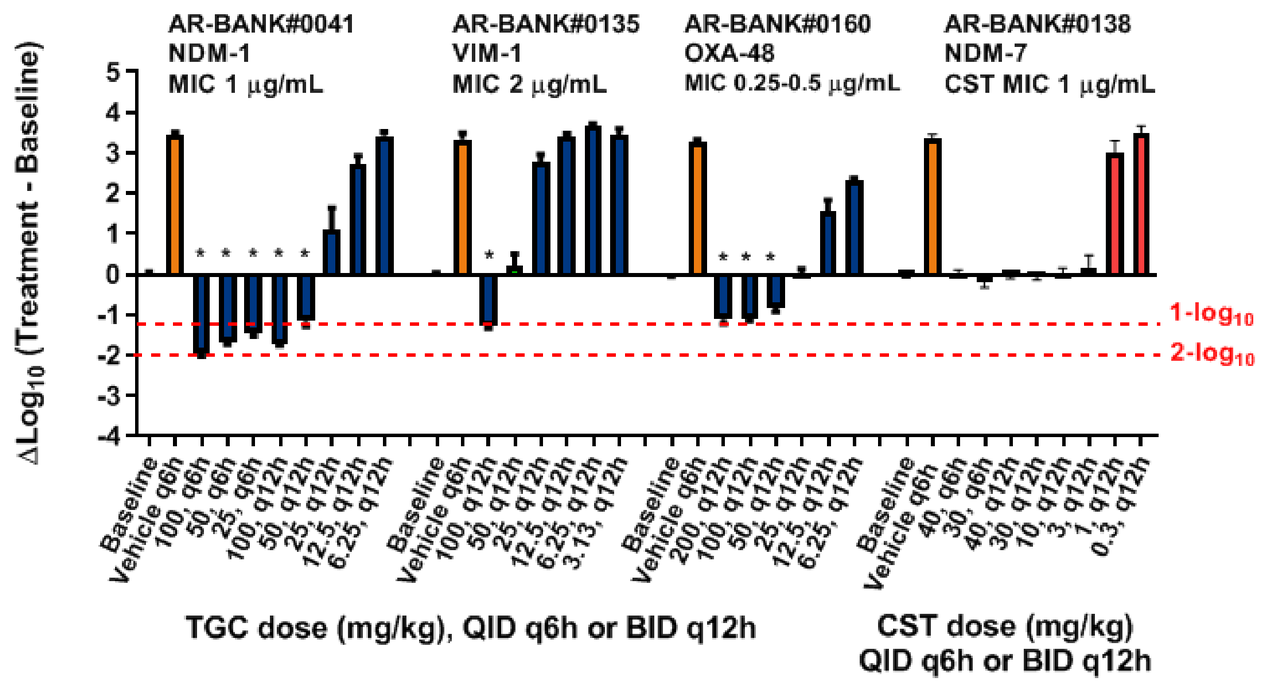
Results

Inoculum, 2 h baseline, and 26 h counts

Strain	Notable MMR	Inoculum count	N	Baseline counts 2 h (CFU/g)	Final counts 26 h (CFU/g)	Log Δ(CFU/g thigh)
				Mean ± SEM	Mean ±SEM	Δ(26 - 2 h)
ATCC 43816		10^4	5	$2 \pm 0.3 \times 10^5$	$4 \pm 0.1 \times 10^9$	4.2
AR-BANK#0087	SHV-12	10^5	5	$7 \pm 2 \times 10^5$	$8 \pm 0.2 \times 10^7$	2.0
AR-BANK#0098	KPC-2	10^5	5	$1 \pm 0.1 \times 10^6$	$1 \pm 0.5 \times 10^8$	1.9
AR-BANK#0097	KPC-3	10^5	5	$5 \pm 2 \times 10^5$	$2 \pm 0.6 \times 10^8$	2.7
AR-BANK#0041	NDM-1	10^5	5	$2 \pm 0.3 \times 10^6$	$5 \pm 1 \times 10^9$	3.4
AR-BANK#0135	VIM-1	10^6	4	$3 \pm 0.3 \times 10^6$	$7 \pm 2 \times 10^9$	3.3
AR-BANK#0160	OXA-48	10^5	5	$2 \pm 0.2 \times 10^6$	$3 \pm 0.4 \times 10^9$	3.3
AR-BANK#0138	NDM-7	10^5	5	$1 \pm 0.3 \times 10^6$	$3 \pm 1 \times 10^9$	3.4

Efficacy of Reference Standards: TGC and CST (AR#0138)





Efficacy Summary

Strain ID	Notable MMR	Reference Antibiotic	MIC (µg/mL)	Calculated efficacious dose (mg/kg/12 h)	
				Bacteriostasis	1-$\log_{10}$ kill
ATCC 43816		Tigecycline	0.5	26	43
AR-BANK#0087	SHV-12	Tigecycline	2	77	N/A
AR-BANK#0098	KPC-2	Tigecycline	0.5 - 1	13	43
AR-BANK#0097	KPC-3	Tigecycline	2	23	54
AR-BANK#0041	NDM-1	Tigecycline	1	33	48
AR-BANK#0135	VIM-1	Tigecycline	2	53	79
AR-BANK#0160	OXA-48	Tigecycline	0.25 – 0.5	26	71
AR-BANK#0138	NDM-7	Colistin	1	5.5	N/A

N/A: Not achievable, exceeds tested dose range

Summary and Conclusions

Thigh infection models with seven MDR *Klebsiella pneumoniae* clinical isolates from the FDA-CDC AR Isolate Bank were established. The panel includes representatives of high risk clones associated multiple international outbreaks (ST258, ST147, ST37, ST101), priority multidrug resistance phenotypes, and resistance mechanisms of KPC-, metallo-β-lactamase, and OXA-48 production.

The clinical isolates grew well in thigh tissue of neutropenic ICR mice with an inoculum count of 10^5 CFU/mouse of most strains. The organisms reached a target count of approximately 10^6 CFU/g thigh (~10^6 CFU/mouse) at baseline, 2 h after infection. The baseline counts and 26 h total counts (untreated) were reproducible between different studies (not shown).

Tigecycline, TGC, was selected as the reference drug for benchmarking the models of most strains because 6 of 7 clinical isolates are TGC susceptible (Table).

TGC administration resulted in bacteriostasis with a median dose of 26 mg/kg q12h BID (52 mg/kg 24 h total dose). TGC administration resulted in a 1-$\log_{10}$ reduction in counts relative to baseline with five of six clinical isolates, median dose of 54 mg/kg q12h BID (108 mg/kg 24 h total dose).

Colistin, CST, was selected as the reference for benchmarking the model with the NDM-7 producing strain, AR-BANK#0138, since this strain has intermediate susceptibility to TGC (Table). CST administration at 5.5 mg/kg BID q12h resulted in bacteriostasis. A 1-$\log_{10}$ reduction in counts relative to baseline was not observed with any colistin dose up to 40 mg/kg administered QID q6h (160 mg/k 24 h dose) (Figure, Table).

Testing with these strains is available through the NIAID Preclinical Testing Services or through Pharmacology Discovery Services. Detailed protocols will be supplied upon request.

References

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