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A Partner Lab of Eurofins Discovery

Development of a Murine Model of *Pseudomonas aeruginosa* Lung Infection

Presented at, Advancing Animal Models for Antibacterial Drug Development Workshop

U.S. Food and Drug Administration, White Oak MD
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NIAID Mission

- **Mission: streamline the discovery of therapeutics for *P. aeruginosa***
- ***P. aeruginosa* is a leading pathogen that causes VABP and HABP**
- **Carbapenem resistant *P. aeruginosa* (CRPA) infections have few treatment options**
- **Related projects**
  - **Conduct testing services to evaluate therapeutic candidates**
  - **Developed mouse thigh and lung infection models with MDR isolates**
  - **Generated PK/PD tutorials with example studies of standard drugs**
  - **Protocols and example data are available to the drug discovery community**

P. aeruginosa Mouse Lung Infection Model

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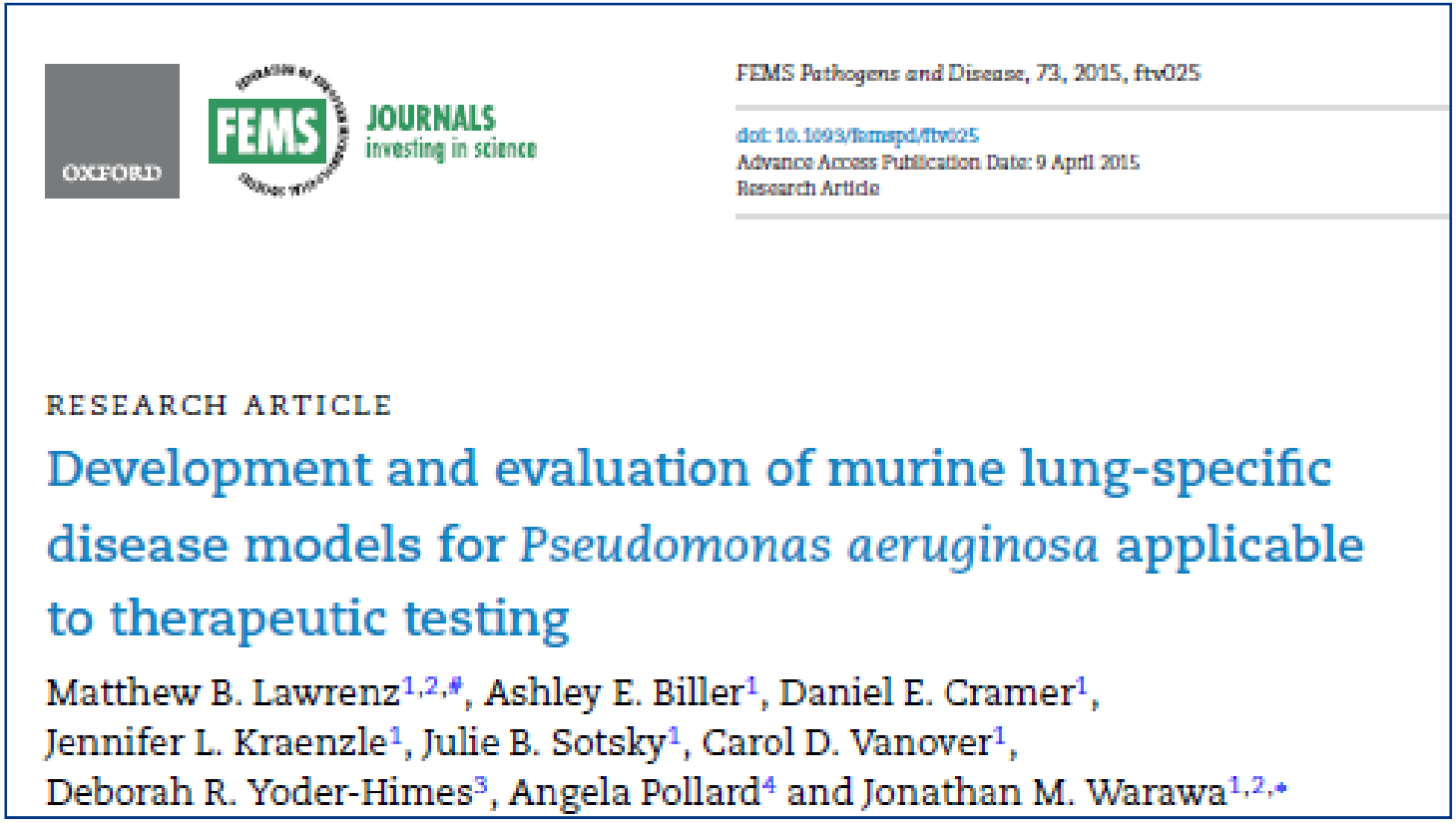
Model features

- Correlate infection mortality with pathogen burden, dissemination and tissue pathology
- Measured endpoints: mortality onset, microbial burden, tissue pathology
- An extended infection period (target ≥ 48 h)
- Compare intranasal (IN) and intratracheal (IT) infection routes
- Conduct with MDR isolates, CDC & FDA AR-BANK

Approach

- Host: persistently neutropenic mice
- Development steps
  - Inoculum optimization
  - Characterize the natural history of infection
  - Benchmark with approved antibiotics

Approach Reference



FEMS Pathogens and Disease, 73, 2015, ftv025

doi: 10.1093/femspd/ftv025
Advance Access Publication Date: 9 April 2015
Research Article

RESEARCH ARTICLE

Development and evaluation of murine lung-specific disease models for *Pseudomonas aeruginosa* applicable to therapeutic testing

Matthew B. Lawrenz[1,2,#], Ashley E. Biller[1], Daniel E. Cramer[1], Jennifer L. Kraenzle[1], Julie B. Sotsky[1], Carol D. Vanover[1], Deborah R. Yoder-Himes[3], Angela Pollard[4] and Jonathan M. Warawa[1,2,*]

FEMS Pathogens and Disease, 73, 2015 ftv025

P. aeruginosa isolates
CDC & FDA AR Isolate Bank

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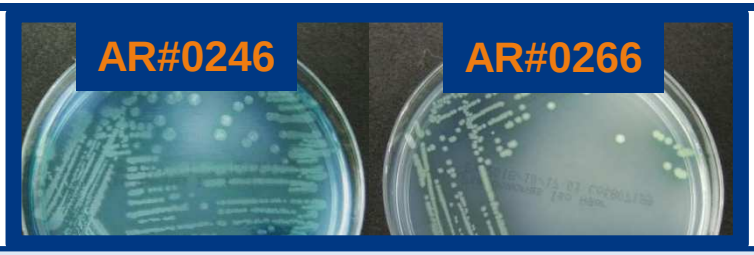
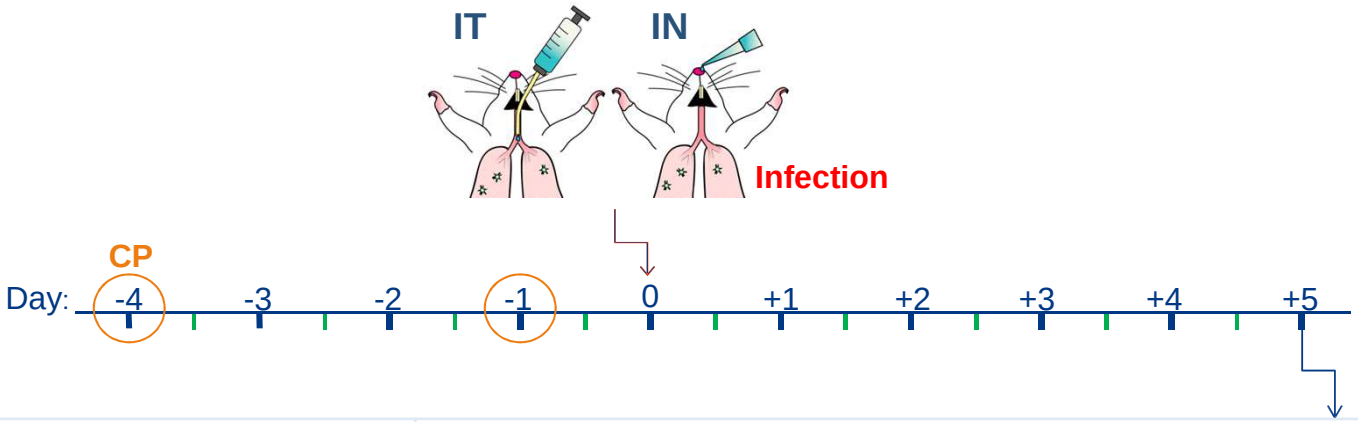


	AR-BANK#0246 CRPA, NDM-1	AR-BANK#0266 CRPA
Antibiotic susceptibility		
Notable AMR gene*	NDM-1	None Detected
Carbapenems	R	R
Piperacillin/tazobactam	R	I
Aztreonam	R	S
Ceftazidime/avibactam	CAZ-R, AVYCAZ-R	CAZ-R, AVYCAZ-S
Amikacin	R	S
Ciprofloxacin, Levofloxacin	R	R
Colistin	S	S
Virulence determinants		
Produce pyocyanin and alginate, motile		

*Genomic information from CDC & FDA AR-Bank

Inoculum Optimization



Mice	Male and Female CD-1 6 weeks
Neutropenia	**CP** 150 mg/kg D-4, 100 mg/kg D-1
Inoculum	Titration
Observations	body temp 6 h intervals, and body weight daily
Humane endpoints	4°C body temp Δ, 20% weight loss, moribund, and diarrhea

- Temperature transponders were used for temperature reads

IPTT300 Bio Medic Data Systems

Mouse figure: van Erp EA *et al.* Viruses 2019, 11(6), 508

Inoculum Optimization
AR-BANK#0246 NDM-1

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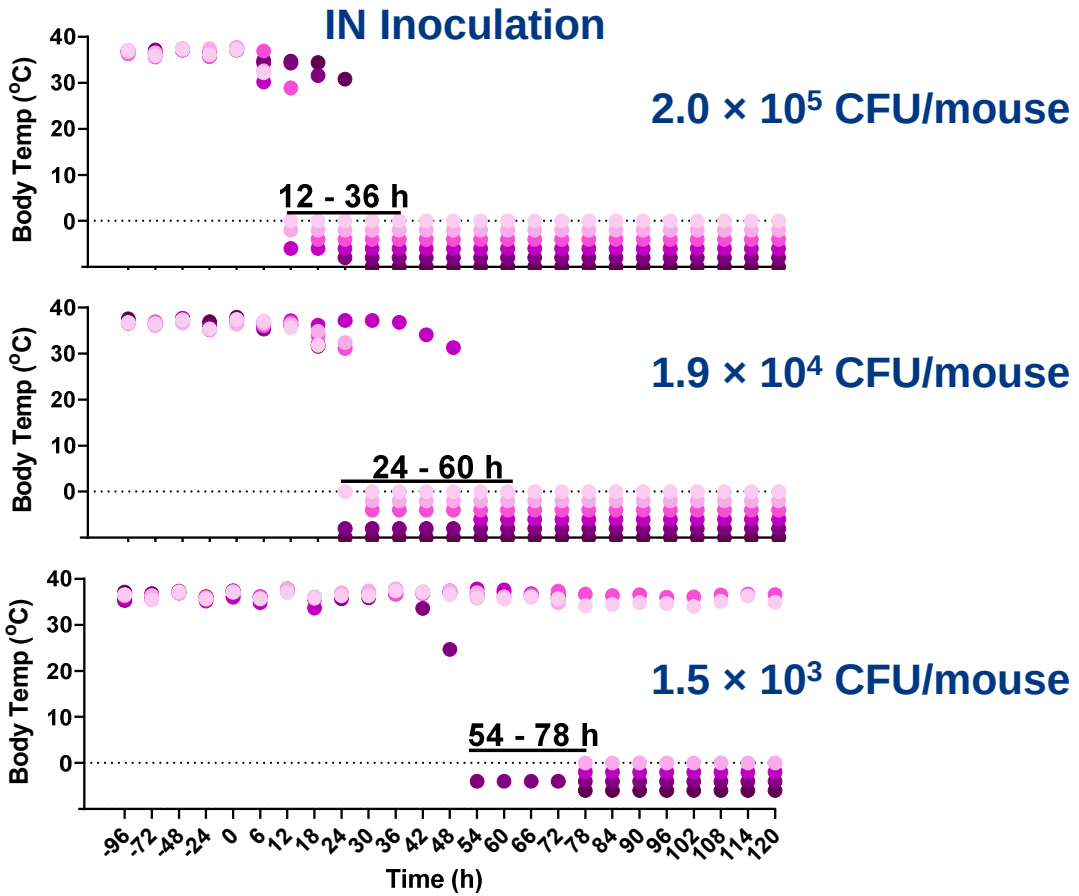
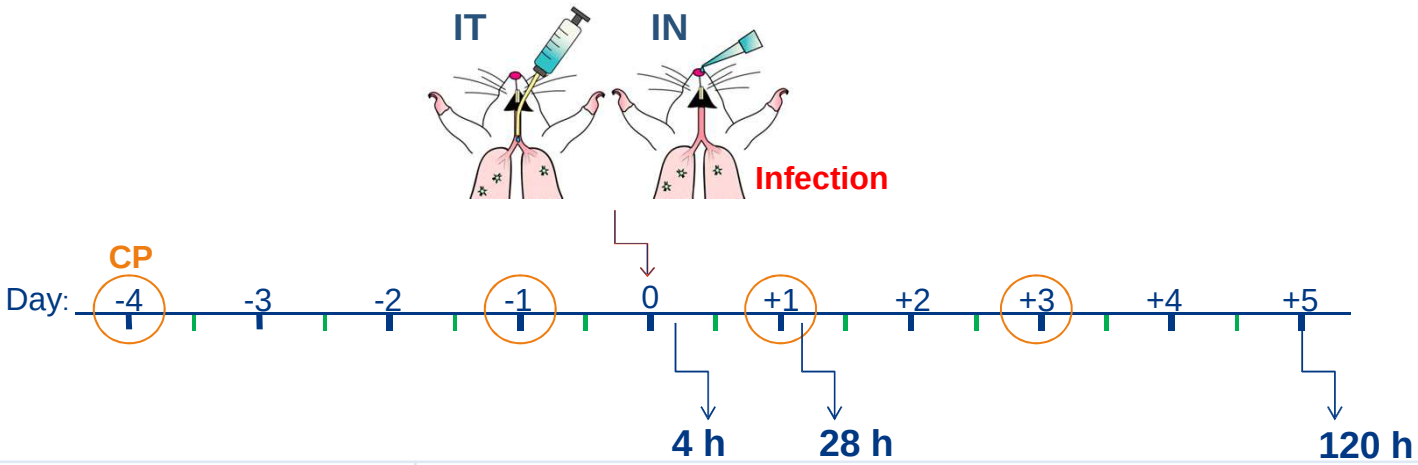


- Iterative titration studies were conducted for each strain
- Body temperature telemetry was highly effective for humane terminal endpoints
- Persistent neutropenia was added to subsequent studies to prevent immune recovery
- Male mice only were selected for subsequent studies to minimize variables

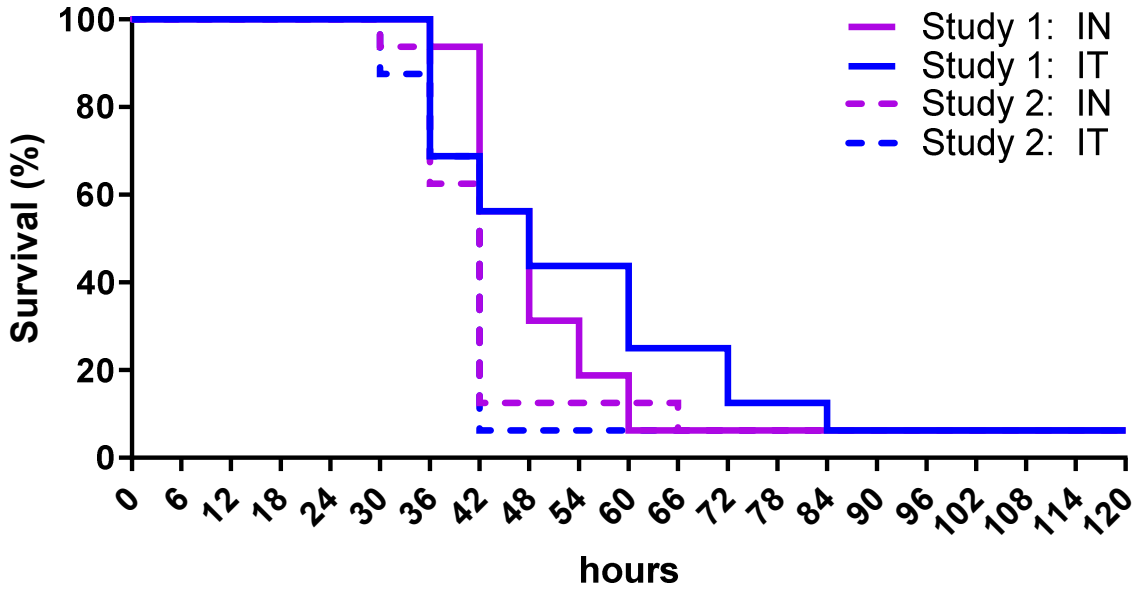
Natural History of Infection



Mice	Male CD-1 6 weeks
Persistent neutropenia	CP 150 mg/kg D-4, 100 mg/kg D-1, D+1, D+3
Target inoculum	#0246 1×10^3; #0266 3×10^3
Observations	body temp 6 h, body weight daily
Humane endpoints	4°C body temp Δ, 20% weight loss, moribund, and diarrhea
Scheduled sacrifice	4 h, 28 h, or 5D post infection
Measurements	mortality onset (h) bacterial CFU/g lung and spleen lung gross pathology and histopathology

Persistent neutropenia: Gumbo T, Drusano GL, Liu W, et al. *Antimicrob Agents Chemother*. 2006;50(11):3695–3700. doi:10.1128/AAC.00507-06
Mouse diagram: van Erp EA *et al.* Viruses 2019, 11(6), 508

Natural History of Infection
AR-BANK#0246 NDM-1



	Study 1	Study 2
Inoculum count (target 1 × 10^3)	8.8 x 10^2	1.6 x 10^3
Mean survival time (h)	IN 46 ± 5 IT 50 ± 6	IN 40 ± 6 IT 39 ± 6

Natural History of Infection
AR-BANK#0246 NDM-1

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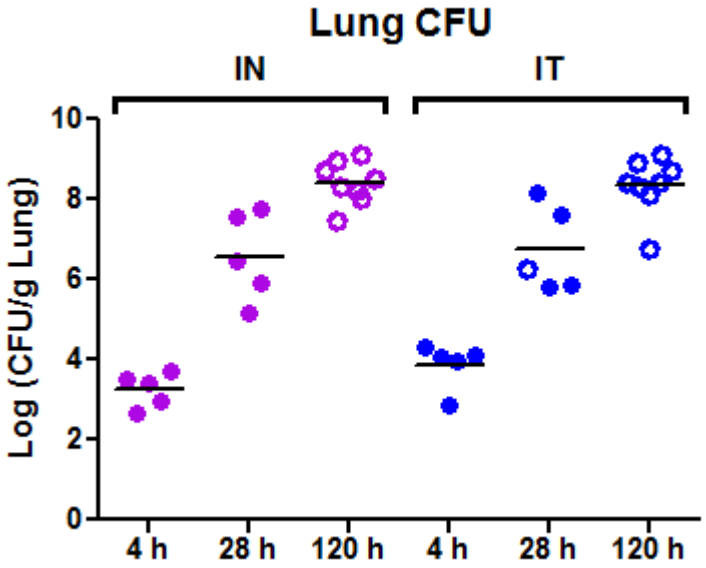
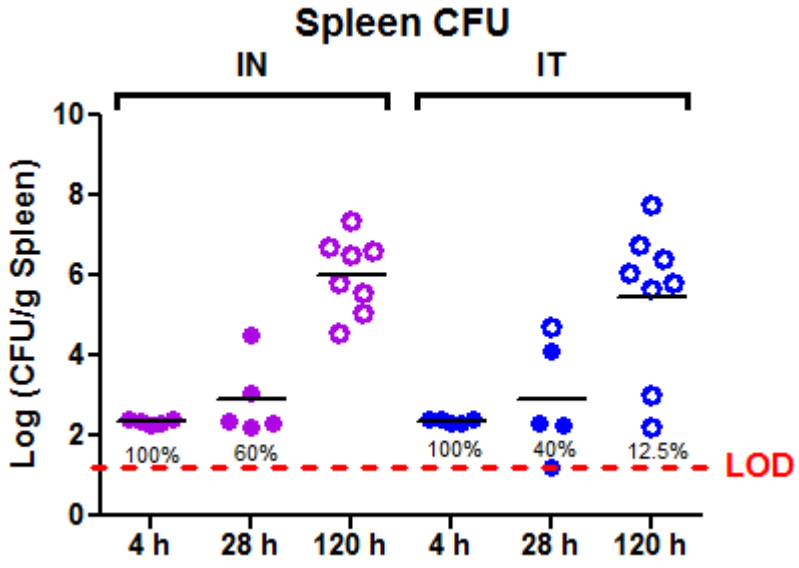
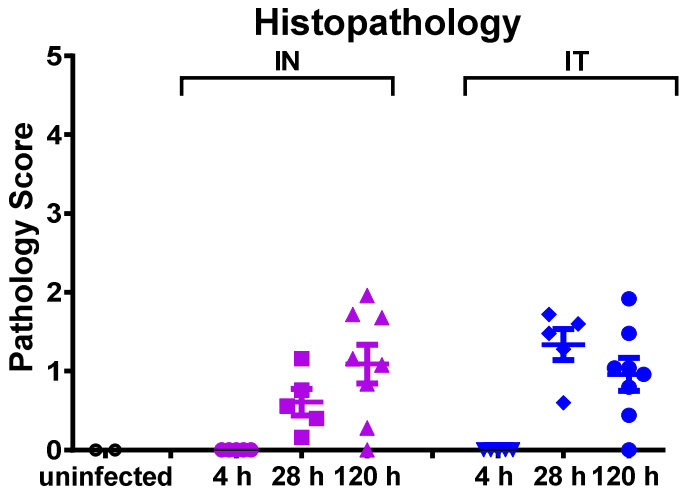
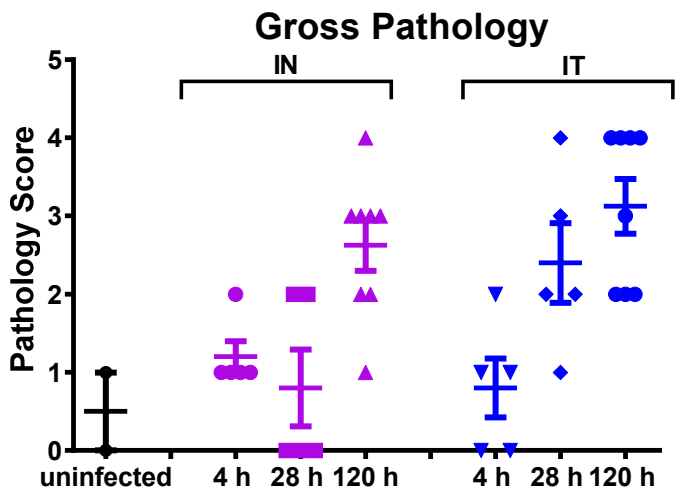


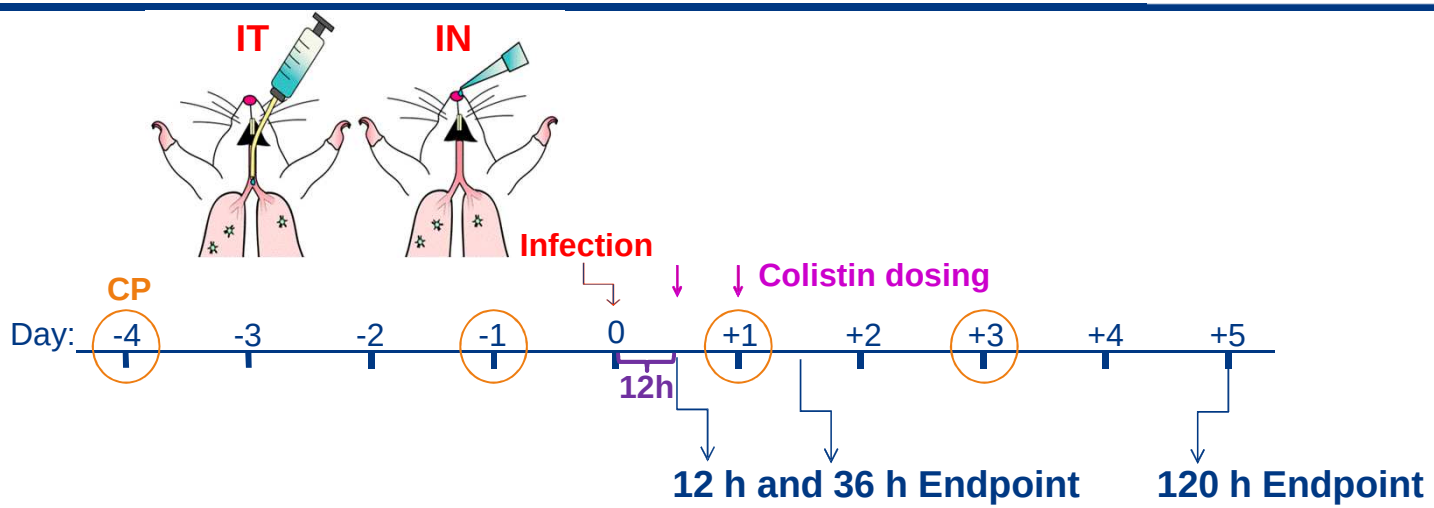


● mice sacrificed at scheduled time points; ○ mice sacrificed at mortality onset





Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)



Mice:	Male CD-1 6 weeks
Persistent neutropenia	CP 150 mg/kg D-4, 100 mg/kg D-1, D+1, D+3
Target Inoculum	1×10^3 CFU
CST dosing	Start 12 h after infection, 30, 20 and 10 mg/kg BID q12h, one day
Humane endpoints	4°C body temp Δ, 20% weight loss, moribund, diarrhea
Scheduled sacrifice times	12 h, 36 h, or 5 D post infection
Measurements	time to mortality onset bacterial counts in lung and spleen lung gross pathology and histopathology

Mouse diagram: van Erp EA *et al.* Viruses 2019, 11(6), 508

Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

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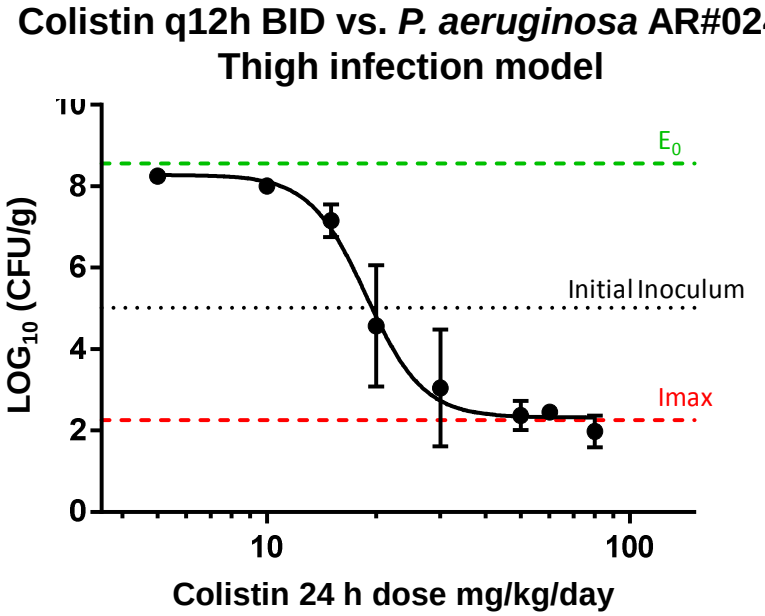
Colistin dose selection

- **Literature data: Dudhani RF, *et al*. 2010 Antimicrob. Agents Chemother. 54:1117**
- **Colistin tolerability in persistently neutropenic mice, 48 h 30 mg/kg q12h**
- **Colistin efficacy, thigh infection model**



Efficacious dose range:
10 - 40 mg/kg q12h BID

HHSN27200005 NIAID PCMID A25
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J. Bulitta, University of Florida team

Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

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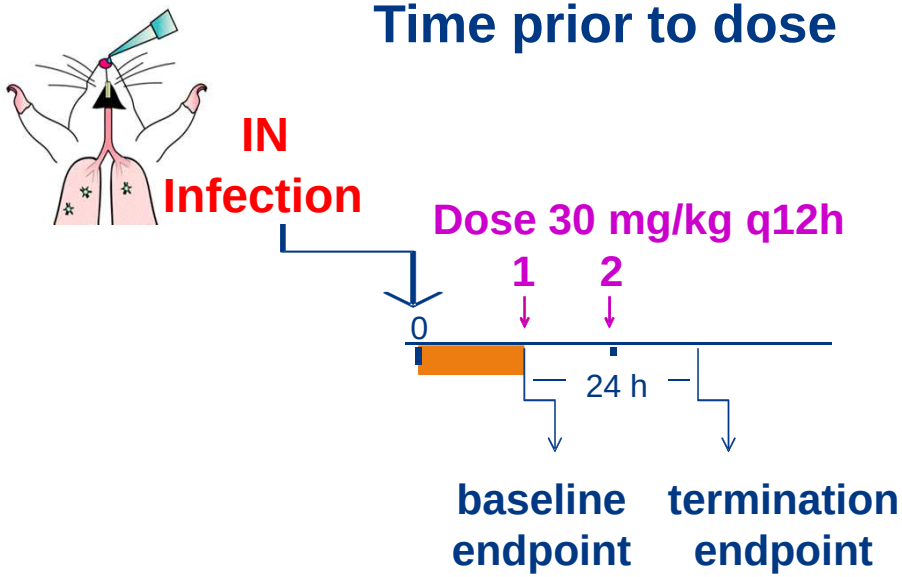

24 h treatment period		
Time of dose 1 ← 12h →	Time of dose 2 ← 12h →	Termination
4 h	16 h	28 h
6 h	18 h	30 h
12 h	24 h	36 h
16 h	28 h	40 h

Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

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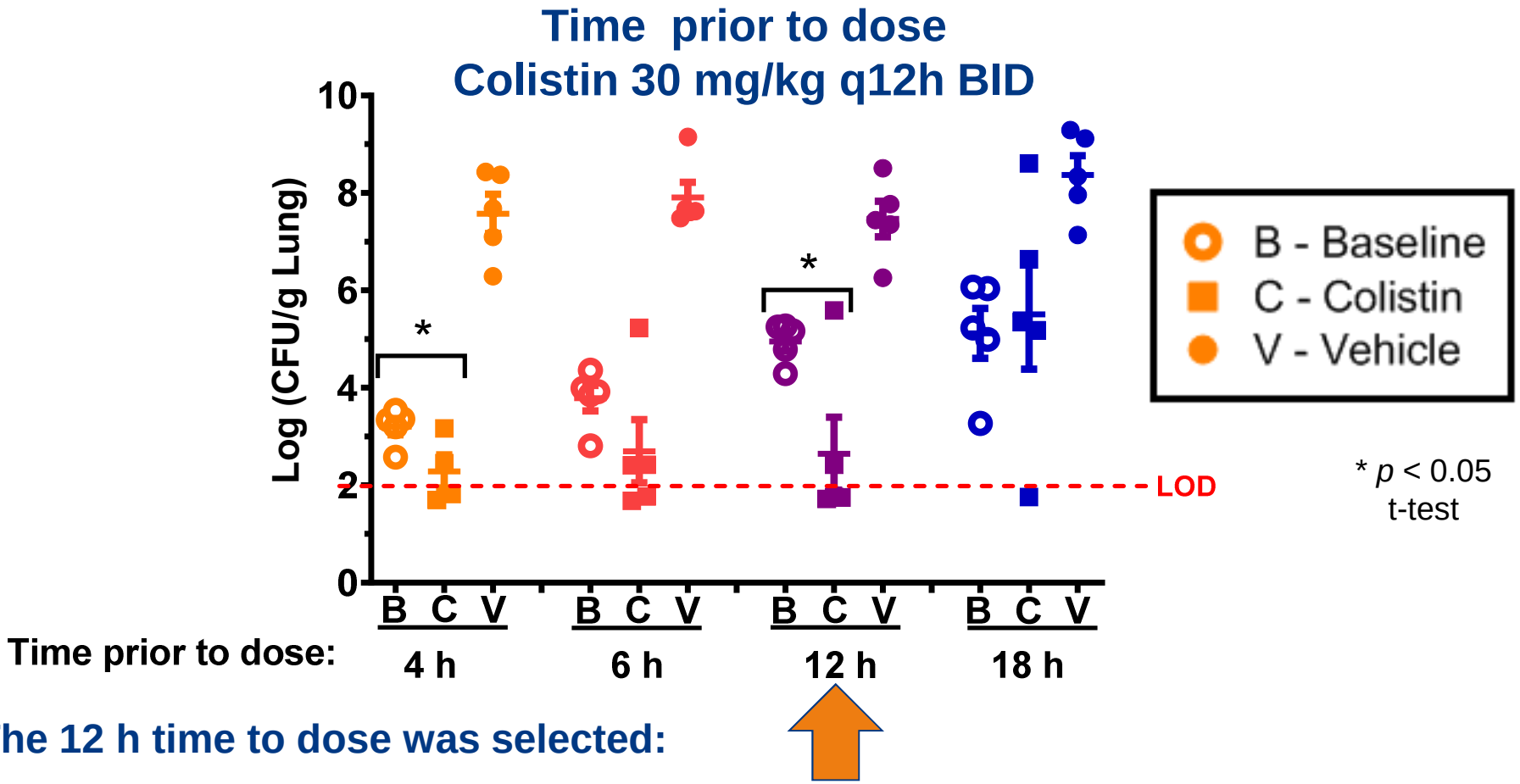

The 12 h time to dose was selected:

(1) ~10^5 CFU/g at baseline

(2) Colistin caused a significant reduction in counts relative to baseline

Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

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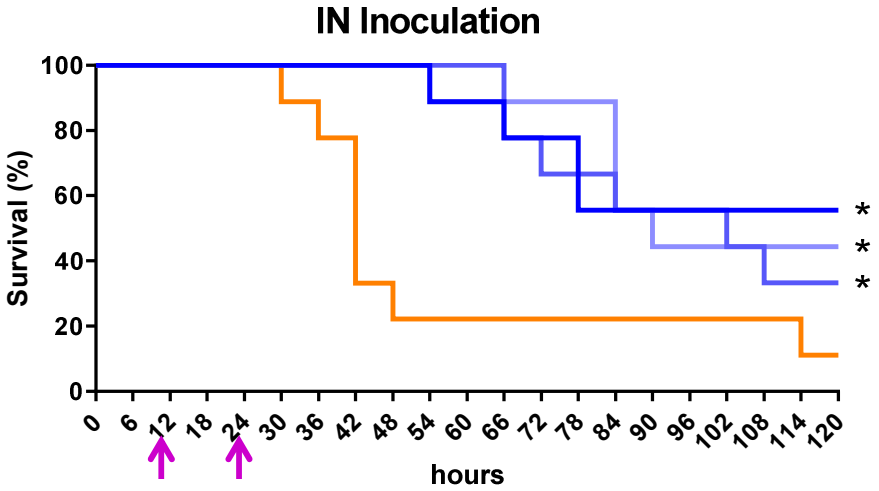
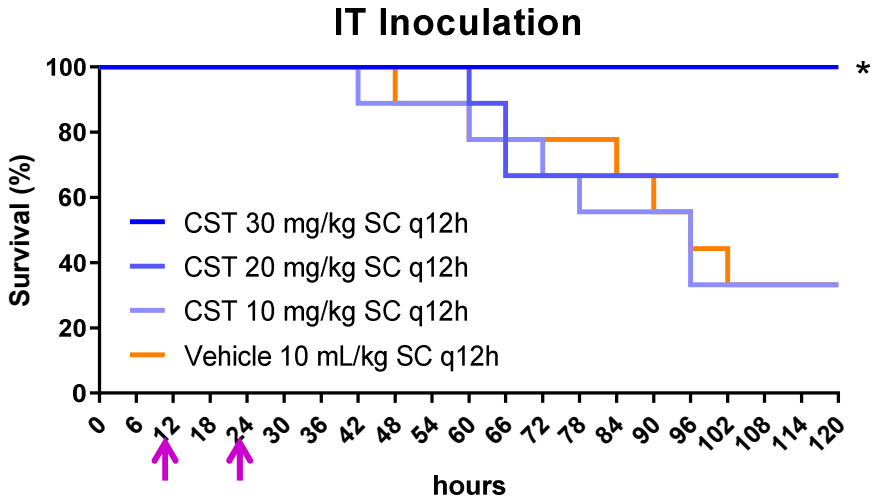
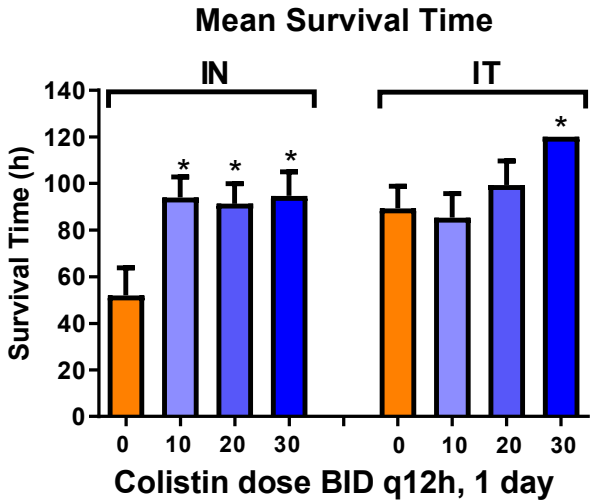
Colistin dosage: BID, q12h, 24 h







Inoculum count: 1.5×10^3
Kaplan Meier: Fisher's exact test
Mean survival time: On-way ANOVA
* $p < 0.05$

Antibiotic Efficacy
AR-BANK#0246 (NDM-1), Colistin (CST)

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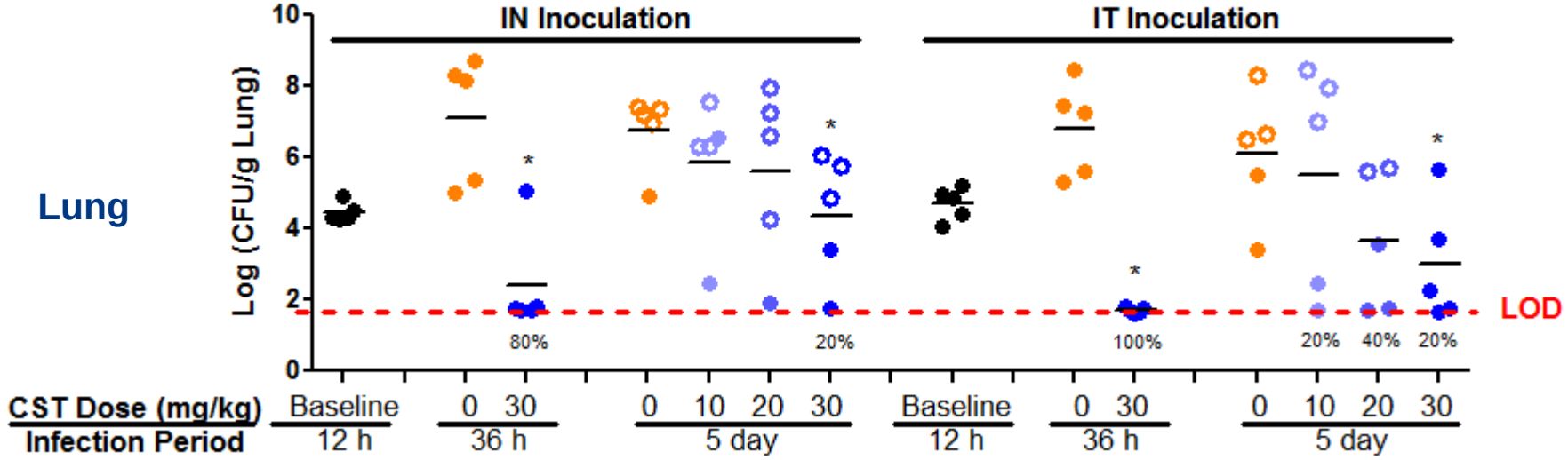
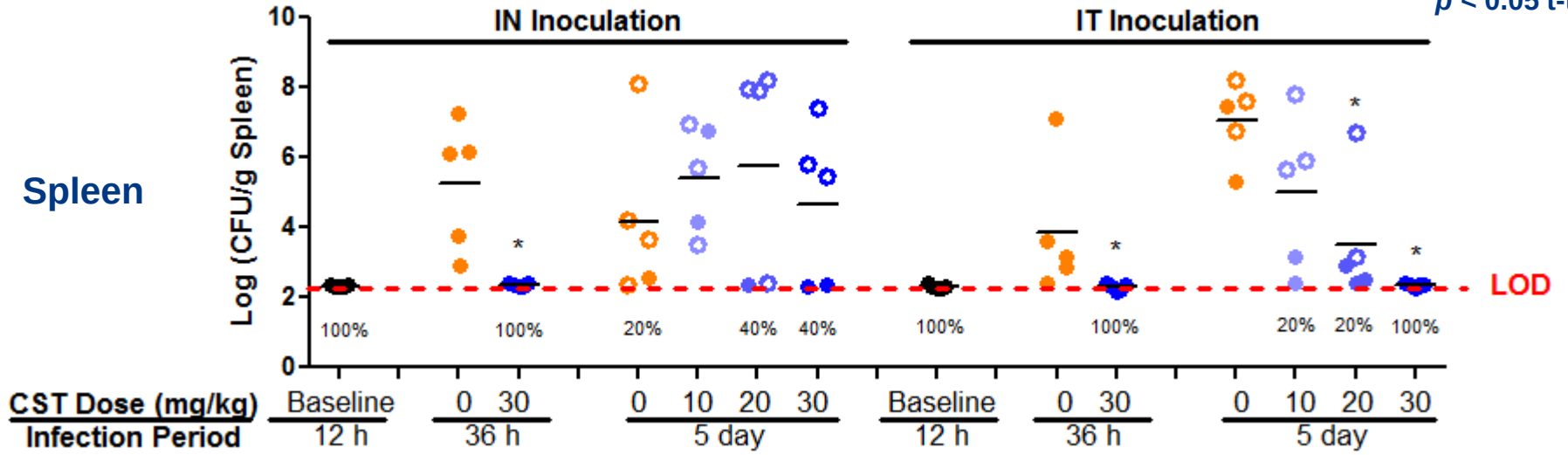
Colistin dosage: BID, q12h, 24 h

Lung



Spleen



* $p < 0.05$ t-test

Summary Across Multiple Studies
FDA & CDC AR-BANK#0246 NDM-1

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Untreated (vehicle treated) mice at the humane endpoint (or 120 h)

	IN Inoculation	IT Inoculation	IN vs IT
Survival (percent)	3.9%	12%	n.s.
Number of animals	77	41	N/A
Survival time (mean, SEM)	42 ± 3	54 ± 5	*
Lung CFU (Log mean, SEM)	7.7 ± 0.2	7.3 ± 0.4	n.s.
Spleen CFU (Log, mean, SEM)	5.5 ± 0.2	5.8 ± 0.4	n.s.
Gross pathology score (SD)	2.5 ± 1.3	3.2 ± 1.3	n.s.
Histopathology score (SD)	1.0 ± 1.1	1.2 ± 1.3	*
Recommended group size	13		

Pooled data from four studies
*** $p < 0.05$**
Survival comparison: Fisher's exact test
Pairwise comparisons: t-test

Summary

- Body temperature monitoring facilitates correlation of disease severity with pathogen burden, dissemination, and tissue pathology
- The approach facilitates selection of time points for dosing and sacrifice
- The isolates were highly virulent in neutropenic mice
- Approved antibiotics (colistin and amikacin) were efficacious
- IN and IT infection yielded similar study results
- Limitation, IT infection yielded more variability: survival time and % mortality

Next Steps and Future Directions

- PK/PD to correlate drug exposure with multiple treatment outcomes
- Establish lung infection models with other pathogens
- Establish lung infection models with immune competent mice

Other Resources, NIAID and PDS

- Drug discovery testing services, 75N93019F00131 (A-32)
- Protocols for murine thigh infection models with MDR isolates, HHSN27200003 (A-25)
- PK/PD tutorial PK/PD tutorials with example studies of standard drugs, HHSN27200003 (A-25)
- Protocols and reports are available to the drug discovery community

Acknowledgments

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Nien-Chen Lee	PDST	Ray Slay	NIAID
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Gin-Feng Lai	PDST	Anna Chiang	NIAID
Jui-Che Chien	PDST	Charles Jackson	NIAID
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