Mouse Model for Testing Therapeutics Against Pulmonary *Pseudomonas* Infection

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272201000033I-1-27200001-1: Task A52
272201000033I-2-27200003-1: Task A72

Development of a Mouse Model for *P. aeruginosa* Infection

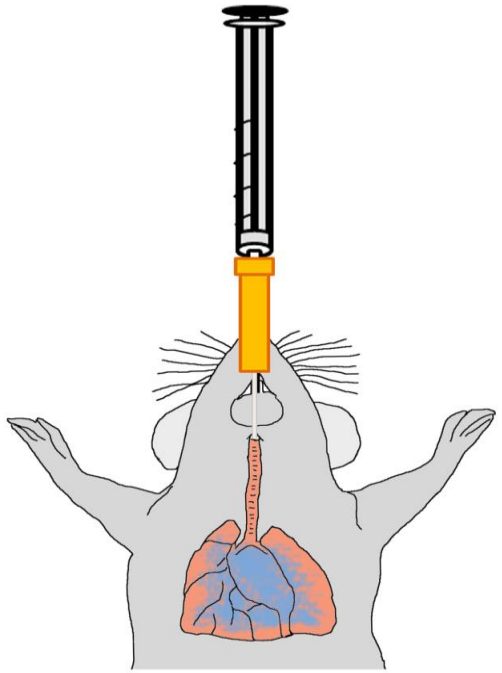
1. Noninvasive instillation of bacteria to establish infection
2. Compare immunocompetent vs. immunocompromised models
3. Identify nonsubjective biometric endpoints
4. Multiple parameters to monitor therapeutic efficacy

References:

1. Lawrenz, M. B., et al. (2015). "Development and evaluation of murine lung-specific disease models for *Pseudomonas aeruginosa* applicable to therapeutic testing." Pathog Dis.
2. Lawrenz, M. B., et al. (2014). "Intubation-mediated intratracheal (IMIT) instillation: a noninvasive, lung-specific delivery system." J Vis Exp(93): e52261.

Intubation-Mediated Intratracheal (IMIT) Instillation

Model for IMIT



1. Intubate with catheter (Otoscope)
2. Insert blunt needle into catheter
3. Instill bacteria (50 µl + 100 µl air)
4. Less than 1 min per mouse

Considerations for inoculation:

1. Intranasal:
   - Upper respiratory track involvement
   - Variability in inoculum reaching lungs
2. Conventional intratracheal
   - Surgery can be technically difficult, slow
   - Potential for blood contamination

Video found at: www.jove.com/video/52261

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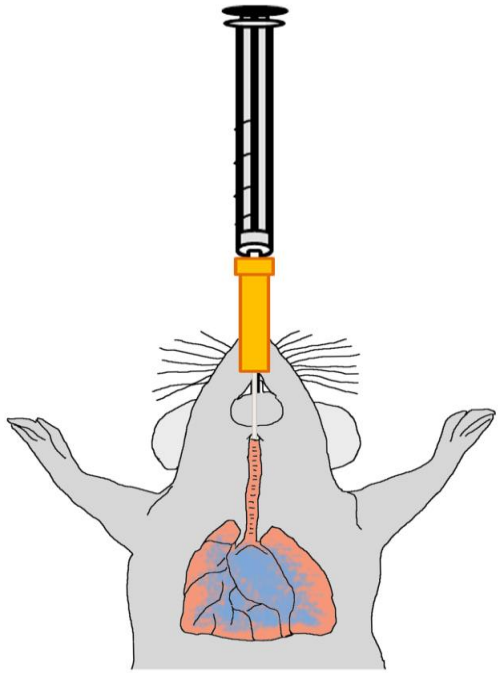
Intubation-Mediated Intratracheal (IMIT) Instillation

Model for IMIT



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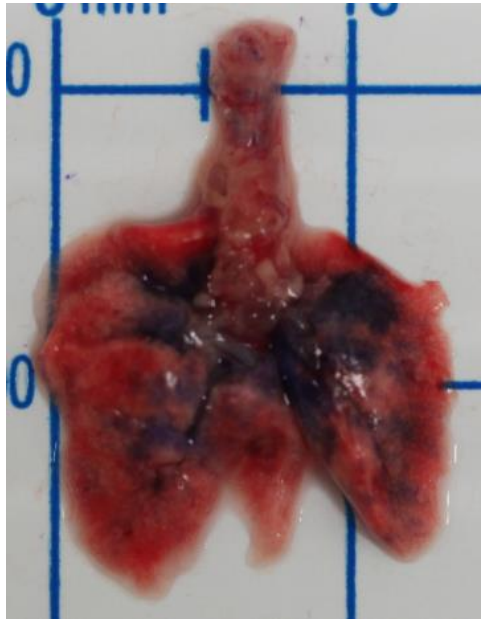
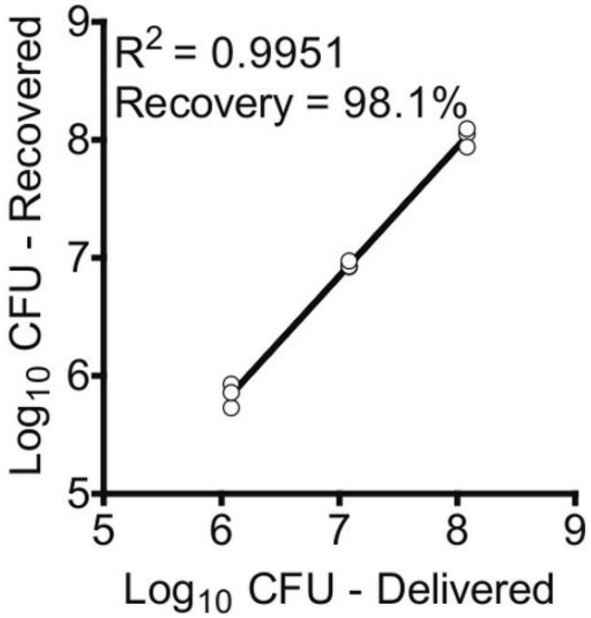
Instillation of dye



1. Broad distribution

Instillation of Pa



1. ~98% efficient delivery of inoculum
2. Reproducible over multiple animals

Video found at: www.jove.com/video/52261

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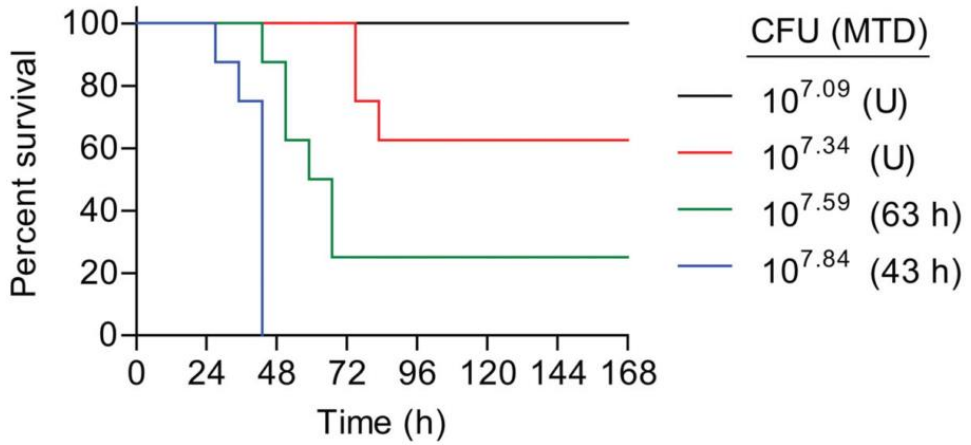
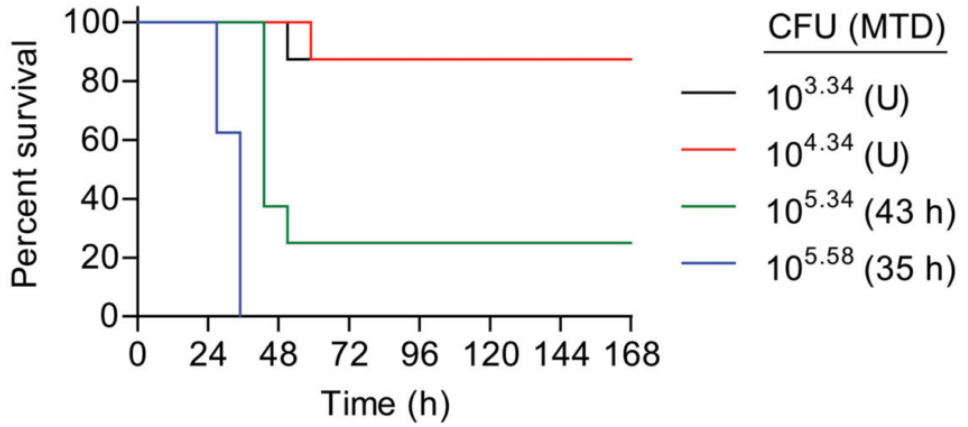
Leukopenia Decreases LD_{50} of *P. aeruginosa*

Balb/C Mice $LD_{50} = 10^{7.44}$ CFU



Leukopenic Balb/C Mice $LD_{50} = 10^{4.56}$ CFU



LD_{50} in leukopenic mice decreased ~760-fold

Because of complications with antibiotic treatment of mice infected with high doses of bacteria, the leukopenic model was chosen as better suited for therapeutic testing (Lawrenz et al. Pathog Dis)

MDR clinical isolate UNC-D; Leukopenia induced by cyclophosphamide treatment (Lawrenz, M. B., et al. (2015). Pathog Dis.)

Leukopenia Decreases LD_{50} of *P. aeruginosa*

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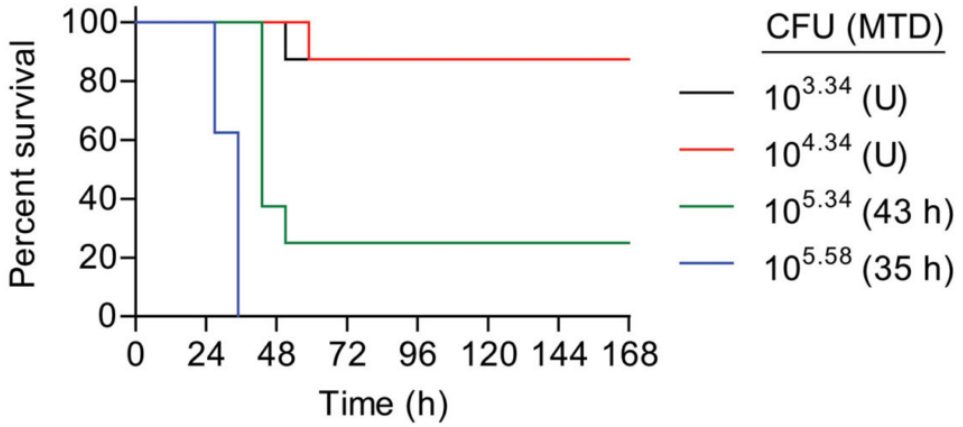

MDR clinical isolate UNC-D; Leukopenia induced by cyclophosphamide treatment (Lawrenz, M. B., et al. (2015). Pathog Dis.)

Establishing Biometric Endpoints

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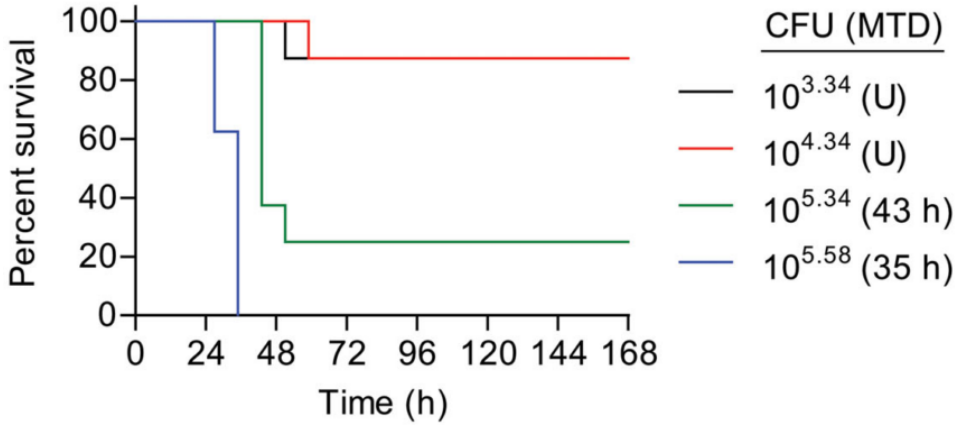
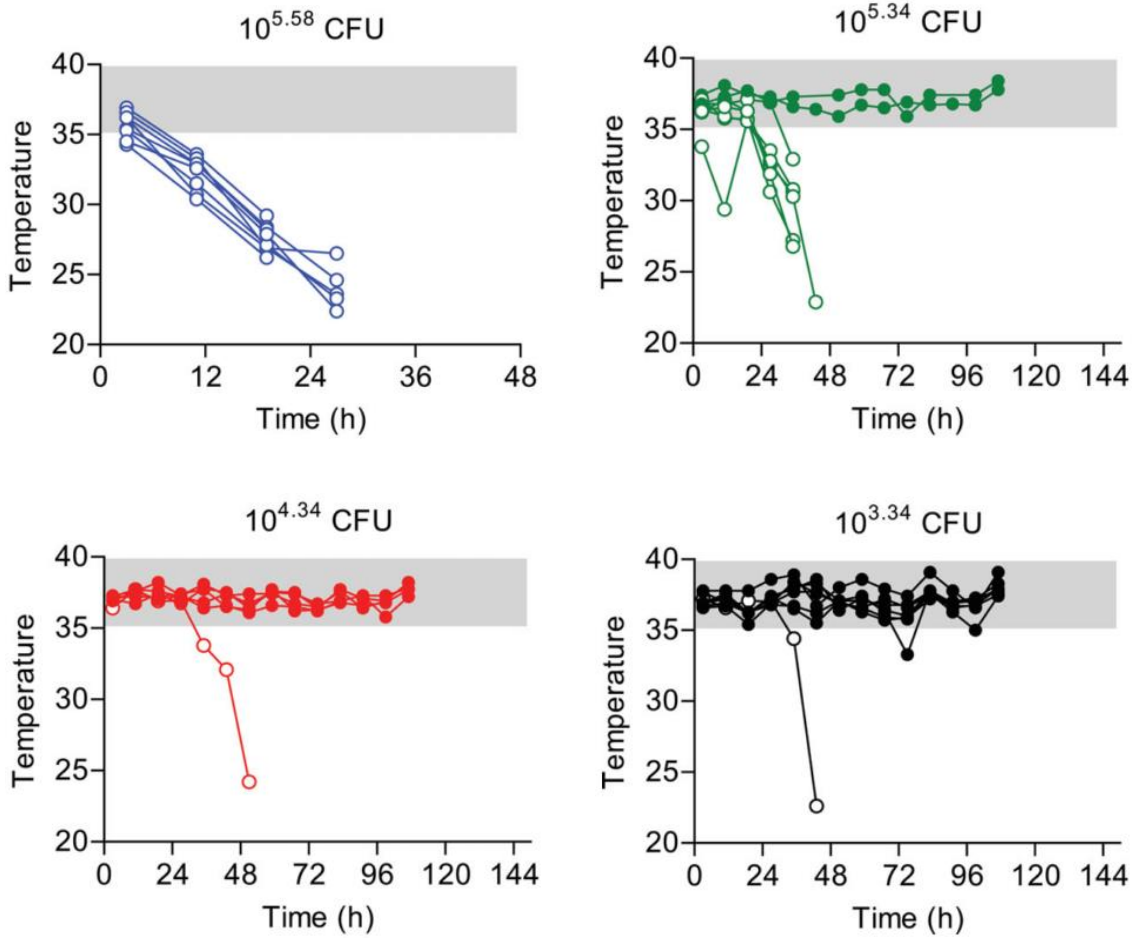


Temperature During Infection



Endpoint Check List:

1. Temperature ≤26.6◦C
2. Heart rate ≤300 bpm
3. Oxygen levels ≤70%
4. Animal has lost its righting reflex

MDR clinical isolate UNC-D; Leukopenia induced by cyclophosphamide treatment (Lawrenz, M. B., et al. (2015). Pathog Dis.)

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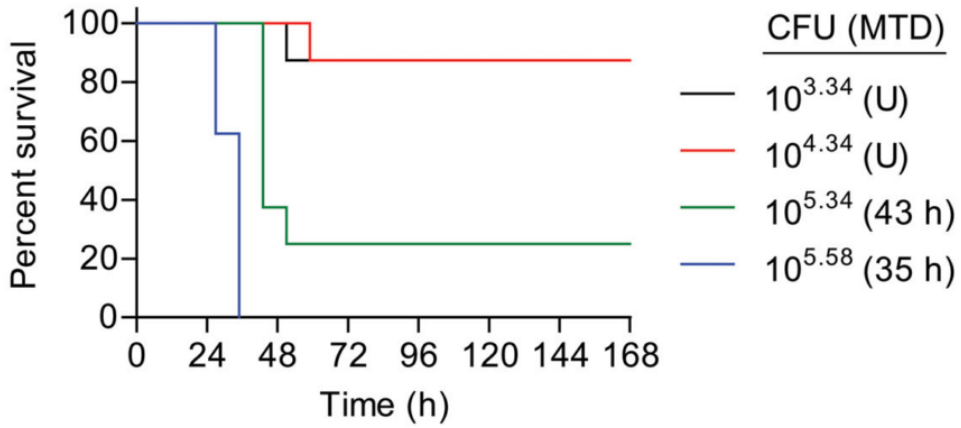
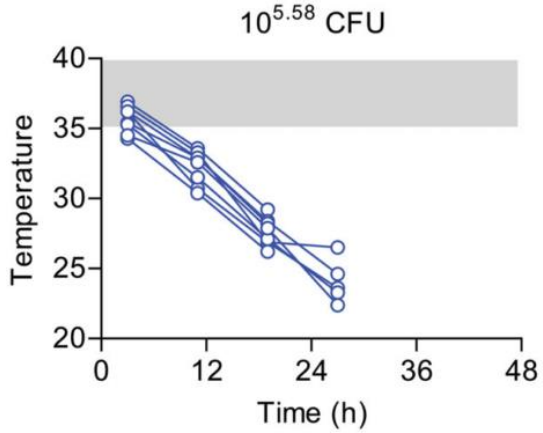
Parameters That Can Measured in the Model

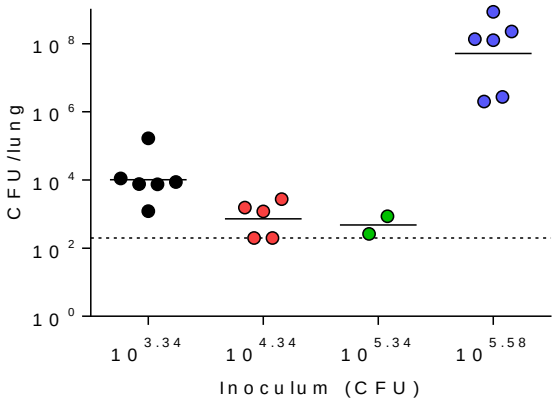
Survival/Mean Time to Death



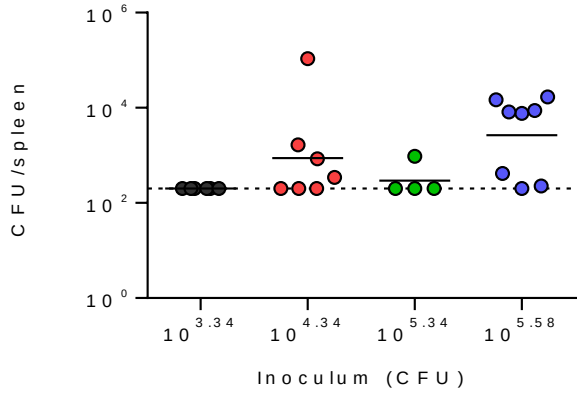
Temperature During Infection



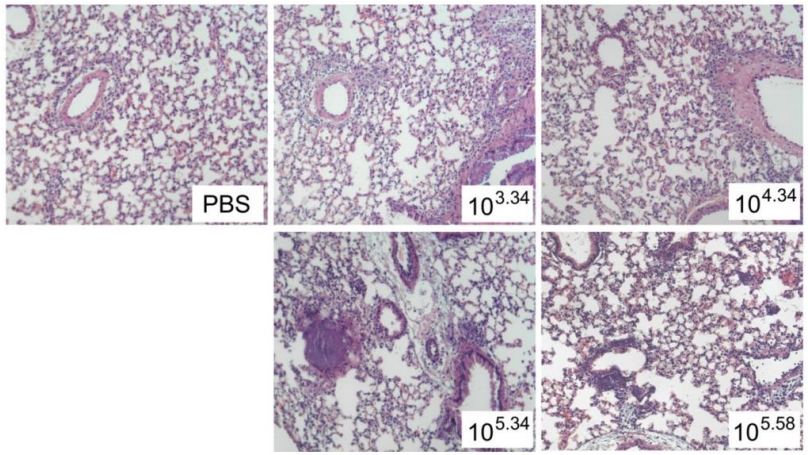
Bacterial Burden - Lungs

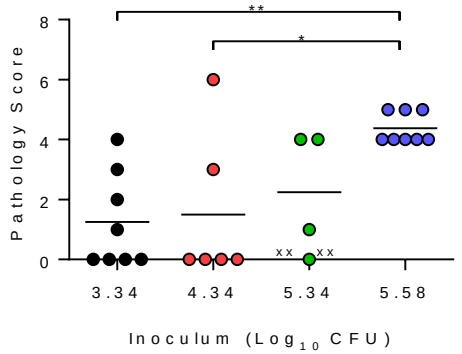


Dissemination - Spleen



Pathology – Lungs





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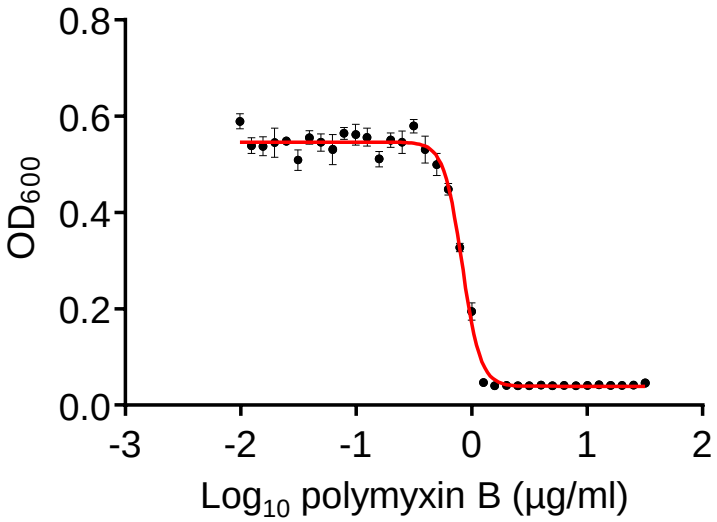
Using the Model for Preclinical Testing of Therapeutics

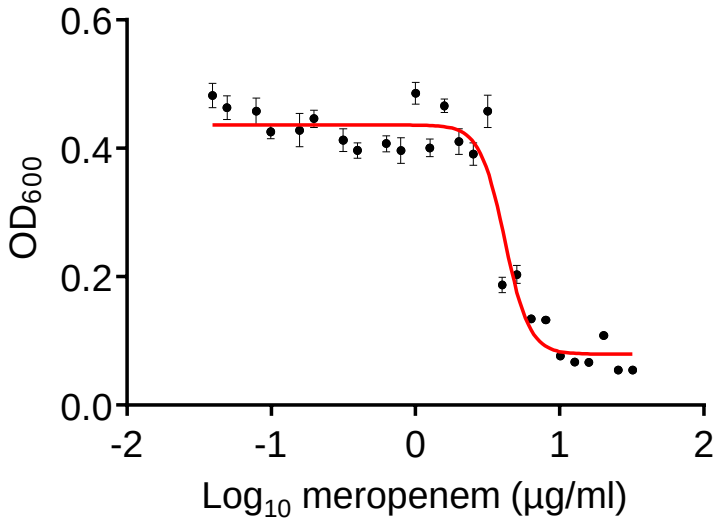
Antibiotics – Polymyxin B vs. Meropenem

Polymyxin B



- EC_{50} = 0.8291 µg/ml
- MIC = 1 µg/ml (sensitive)

Meropenem



- EC_{50} = 4.159 µg/ml
- MIC = 8 µg/ml (intermediate)

MDR clinical isolate UNC-D; Unpublished data.

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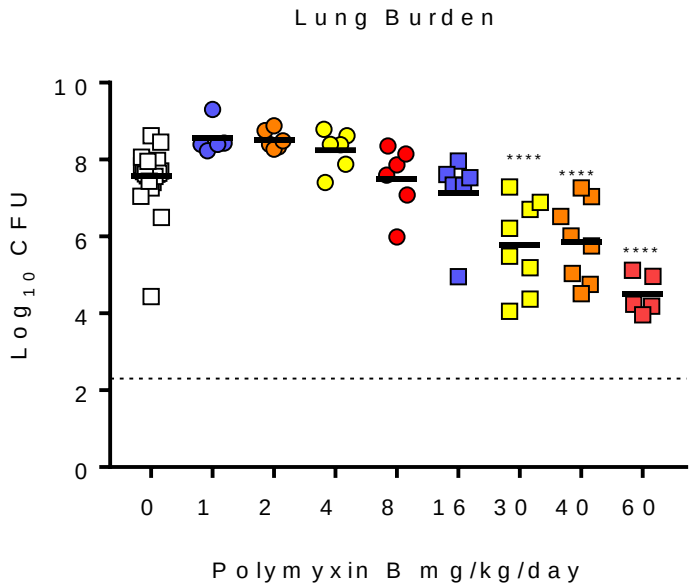
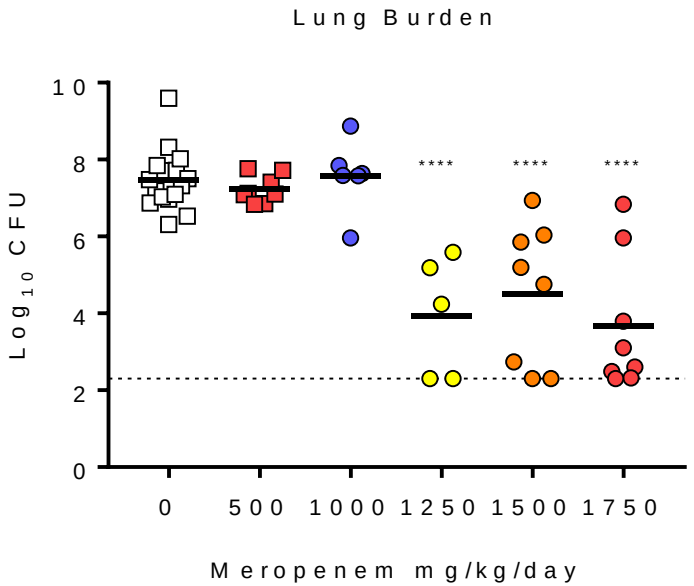
Antibiotics – Polymyxin B vs. Meropenem

Polymyxin B



- Dose dependent inhibition of bacterial numbers in the lungs

Meropenem



- Dose dependent inhibition of bacterial numbers in the lungs
- At LOD in higher doses

MDR clinical isolate UNC-D. Therapy began at 3 h post-infection and continued q8 for 5 days. Continued monitoring for 7 days.

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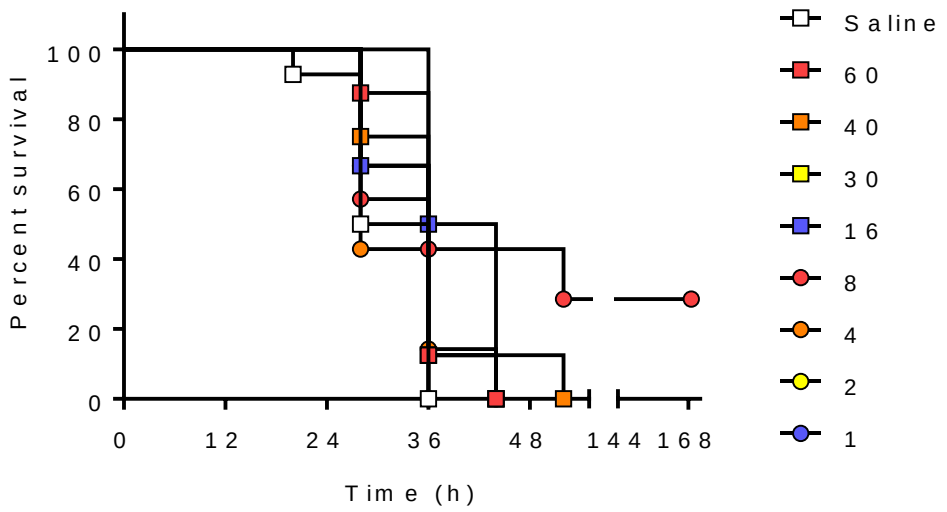
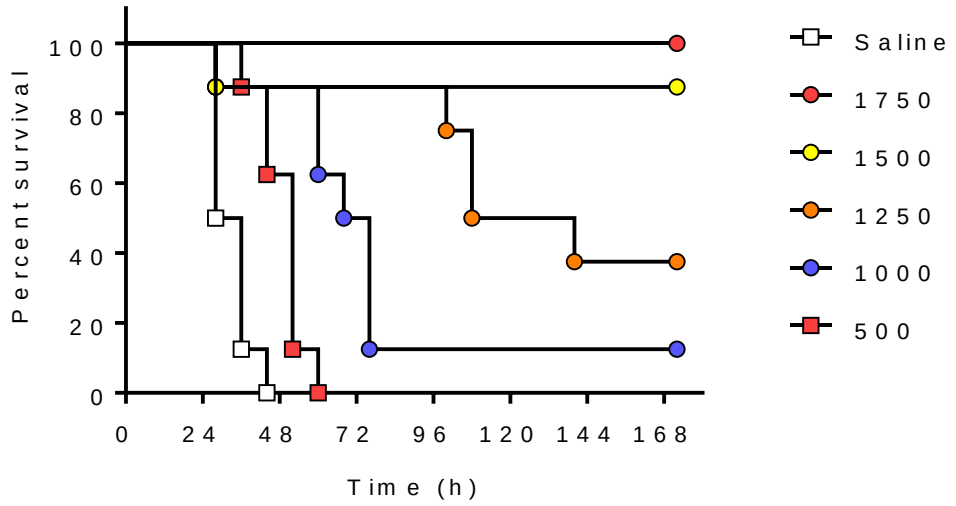
Antibiotics – Polymyxin B vs. Meropenem

Polymyxin B



- No change in survival
- No change in dissemination (data not shown)
- No change in pathology (data not shown)

Meropenem



- Dose dependent increase in MTD
- ED_{50} = 1,258.5 ± 73 mg/kg/day
- Significant decrease in dissemination (data not shown)
- Significant decrease in pathology (data not shown)

MDR clinical isolate UNC-D. Therapy began at 3 h post-infection and continued q8 for 5 days. Continued monitoring for 7 days.

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Using the Model for Preclinical Testing of Therapeutics

Testing novel therapeutics

This model has also proven amenable to:

- Different routes of administration of therapeutics, including but not limited to, subcutaneous and IP injection, intranasal and IMIT instillation, and aerosol delivery.
- Preclinical screening of both monotherapies and combination/adjunct therapies.

To date we have used this model to test 11 different therapeutics, which have included novel antibiotics, small compounds, and biologicals.