Animal models to test the safety and efficacy of novel antibacterials against *Acinetobacter baumannii*

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MARCH 1, 2017

WRAIR
Walter Reed Army Institute of Research
Soldier Health • World Health

Bacterial Diseases Branch
Wound Infections Department
WID
Defeating combat wound infections

Disclaimers

All animal research was IACUC reviewed and performed under an approved protocol and in an AAALACi accredited facility. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition.

The findings and opinions expressed herein belong to the authors and do not necessarily reflect the official policy or position of the WRAIR, the Department of the U.S. Army, the Department of Defense, or the U.S. Government.

Brief Background

WOUND INFECTIONS DEPARTMENT (FOUNDED 2009) AT THE WRAIR:

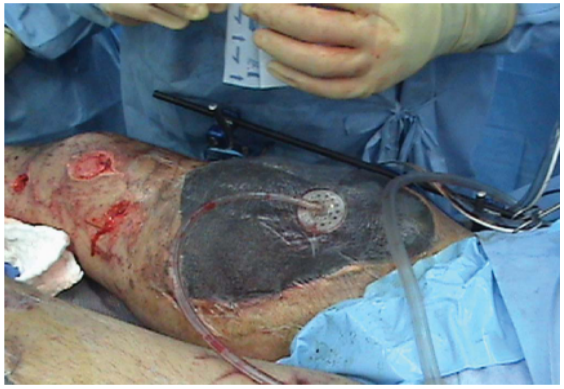
- **THE MISSION** - TO DEFEAT COMBAT-RELATED WOUND INFECTION
- **THE VISION** - CONDUCT REQUIREMENTS-DRIVEN PROGRAMMATIC RESEARCH USING A MULTI-DISCIPLINARY APPROACH TO CHARACTERIZE WOUND PATHOGENESIS AND DEVELOP NOVEL ANTIMICROBIAL COUNTERMEASURES FOR WOUND INFECTIONS OF SERVICE MEMBERS.

Wounded Numbers in OIF + OEF +OND +OIR +OFS (2016)	
WIA	52,352
Deaths from Wounds	1289
Amputees	1645

Sources: DCAS website
Fisher et al. 2015

THE BAD ACTORS – ESKAPEE

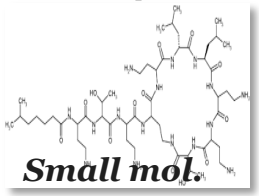
- ***Enterobacter clocae***
- ***Staphylococcus aureus***
- ***Klebsiella pneumoniae***
- ***Acinetobacter baumannii***
- ***Pseudomonas aeruginosa***
- ***Enterococcus faceium***
- ***Escherichia coli***

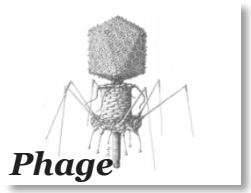


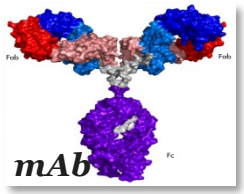
Powell, ET IV, *J Orthop Trauma* 22(10) 2008

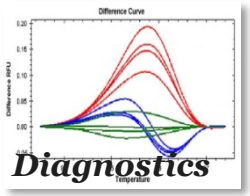
Wound Infections Department - WRAIR

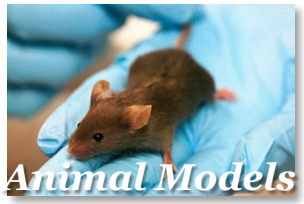
Mission
Defeat combat-related wound infections





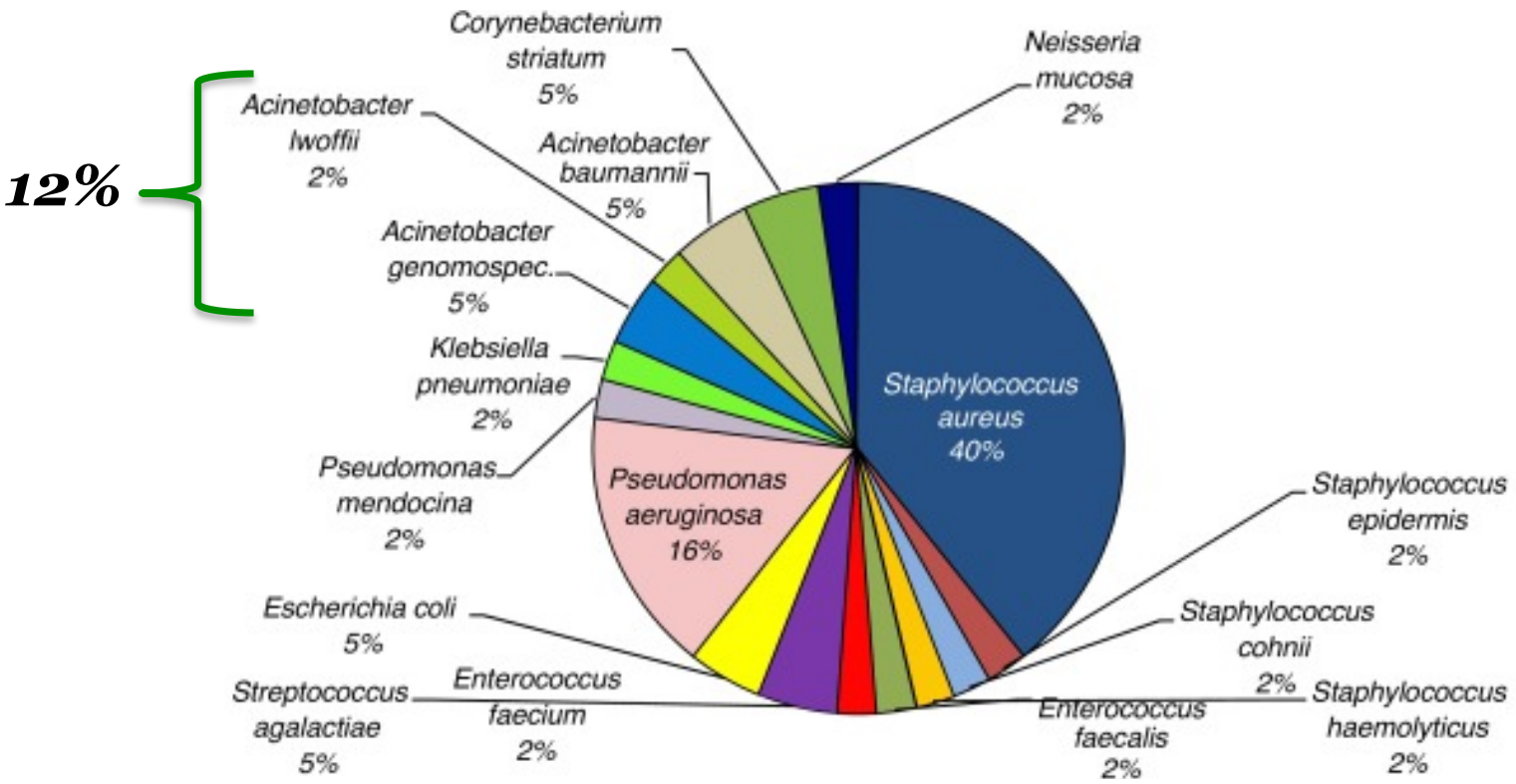






Structured around ***lines of effort***

Microbiome vs. Pathogenesis



Chudobova et al. 2016

Acinetobacter baumannii model development

Three Parts:

1. **Strain selection**

2. **Pulmonary Murine Model of Infection (VAP)**
   A. Model description
   B. Example of use

3. **Wound Models of infection (SSTI)**
   A. Murine model
   B. Possible optimizations to the model
   C. Porcine model

Where to begin?
Strain selection

Strain selection – what do you use as your model strain?

- Clinically relevant – recent isolates
- MDR/XDR – to fit the current problem.
- Genetically amenable – virulence factors
- Cause similar infectious disease in animal models of infection
- More Virulent – enhance therapeutic window
- Can it predict efficacy? – positive controls (known susceptibility)
- Can it be standardized across more than one animal model?

Strain Selection – *Acinetobacter baumannii*

- **Early Literature** – 15-20 years ago
  - Most people use ATCC 17978 and ATCC 19606
    - These strains are 50+ years old
    - Not virulent in animal models
    - No recent evolutionary changes.
    - Not MDR/XDR

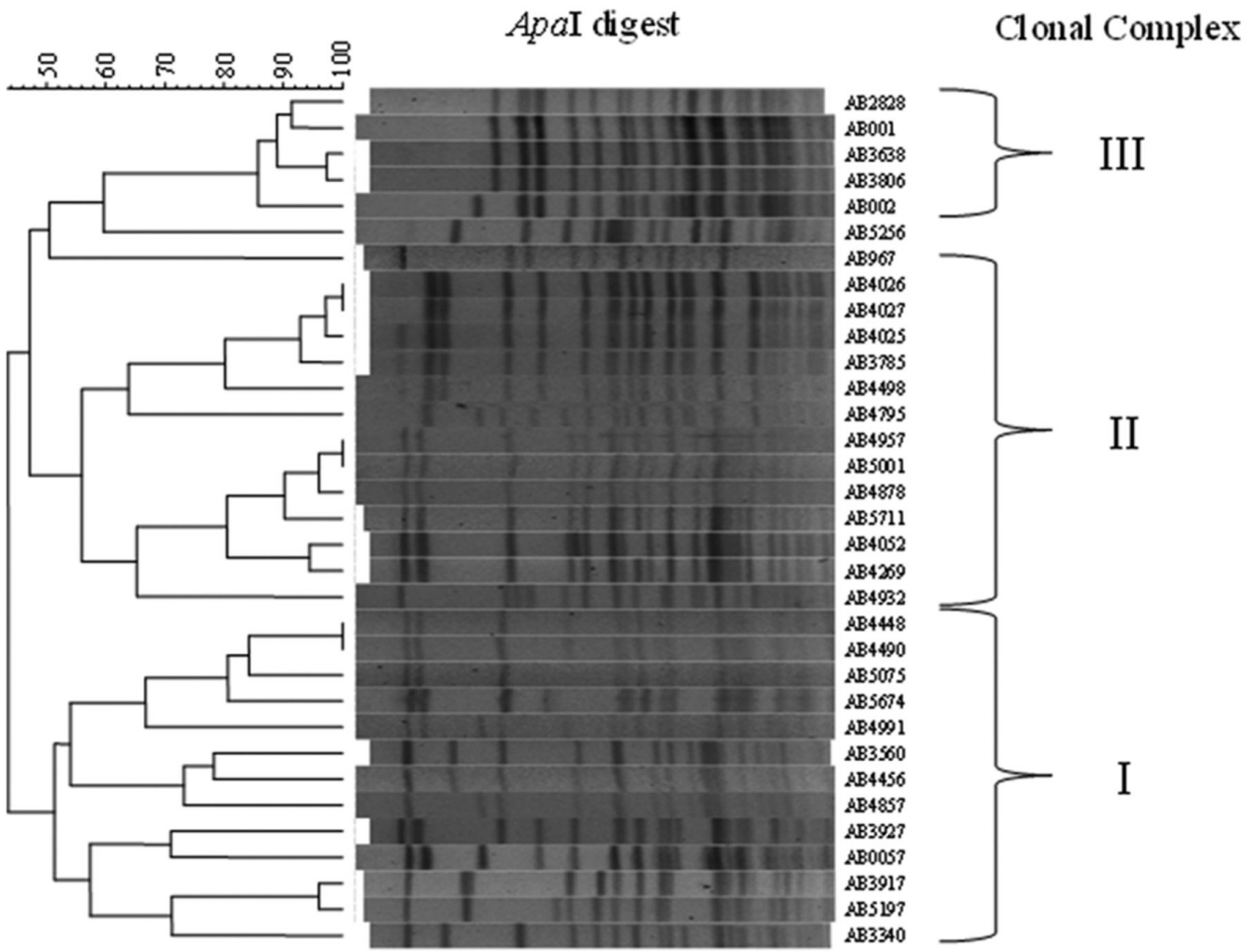
- **Recent Literature** – Last 5-10 years
  - Europeans analyzing clinical isolates for virulence (Eveilard et al. 2010).
  - Clinical isolates
    - Virulent in animal models and genetically tractable – (Russo et al. 2008)
    - Genetically tractable/naturally competent – (Ramirez et al. 2010)

Acinetobacter baumannii strains

SOURCE	FREEZER #	WRAMC PFGE DONE	WRAMC PFT
Blood	3560	Y	1
Blood	5256	Y	1
STS	4957	Y	2
Blood	5001	Y	2
Blood	5711	Y	2
Blood	3785	Y	3
Blood	4498	Y	3
Blood	2828	Y	4
STS	3638	Y	4
STS	3806	Y	4
Blood	3917	Y	4
Warwound	4269	Y	5
STS Bone	4795	Y	6
STS	5197	Y	7
Blood	3340	Y	8
Blood	5674	Y	9
Blood	967	Y	10
Warwound	4878	Y	11
STS	4857	Y	12
Warwound	4991	Y	21
Warwound	4052	Y	23
Sputum	4932	Y	25
Trachasp	4456	Y	27
Warwound	4448	Y	34
Warwound	4490	Y	34
Bone	5075	N	ND
Femur	4025	N	ND
Fibula	4026	N	ND
Femur	4027	N	ND
Tibia	3927	N	ND

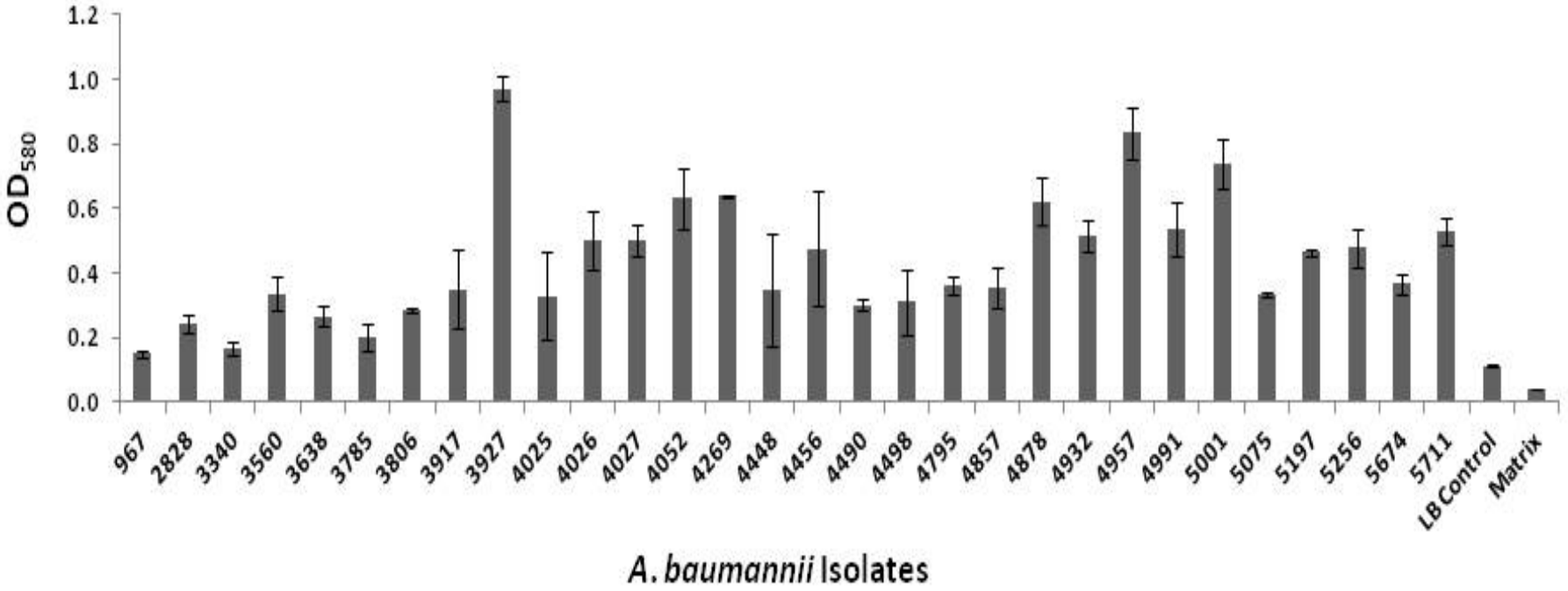
Jacobs et al., 2014, *mBio*

Acinetobacter baumannii strains - PFGE



Anna C. Jacobs et al. mBio 2014; doi:10.1128/mBio.
01076-14

Biofilm Diversity

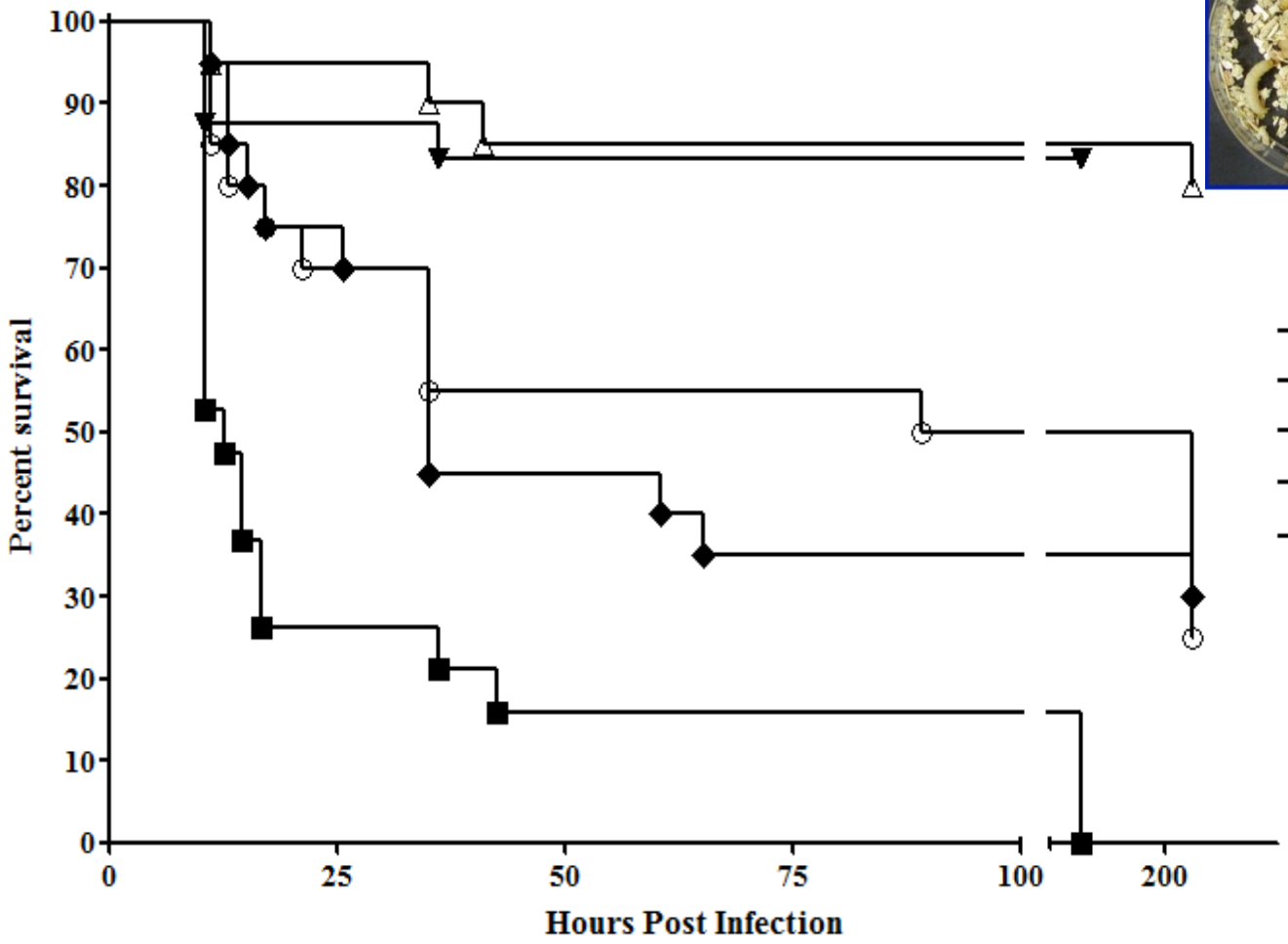


Unpublished data

Motility and Optical Mapping Diversity

McQueary et al. 2012

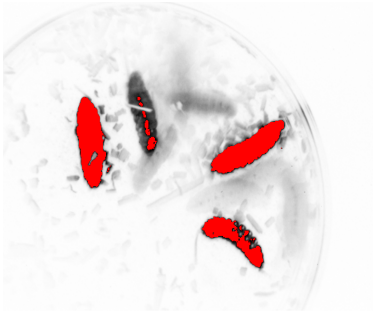
Virulence in *Galleria mellonella*



N=20

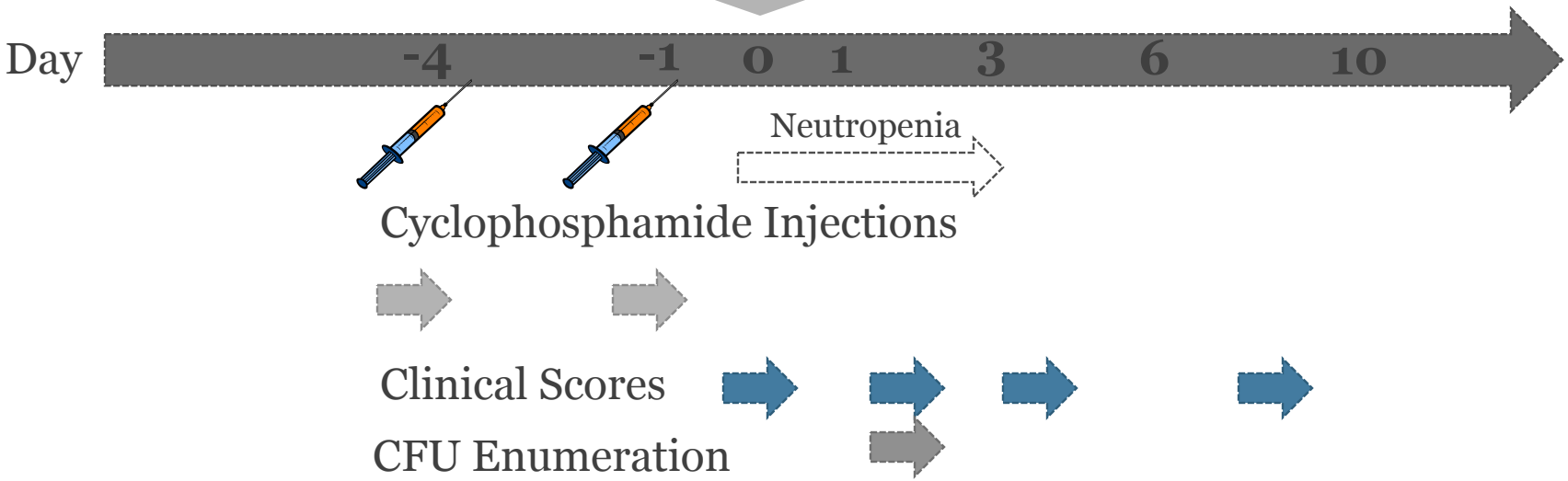
***P* value = .0001**

Mantel – Cox Test

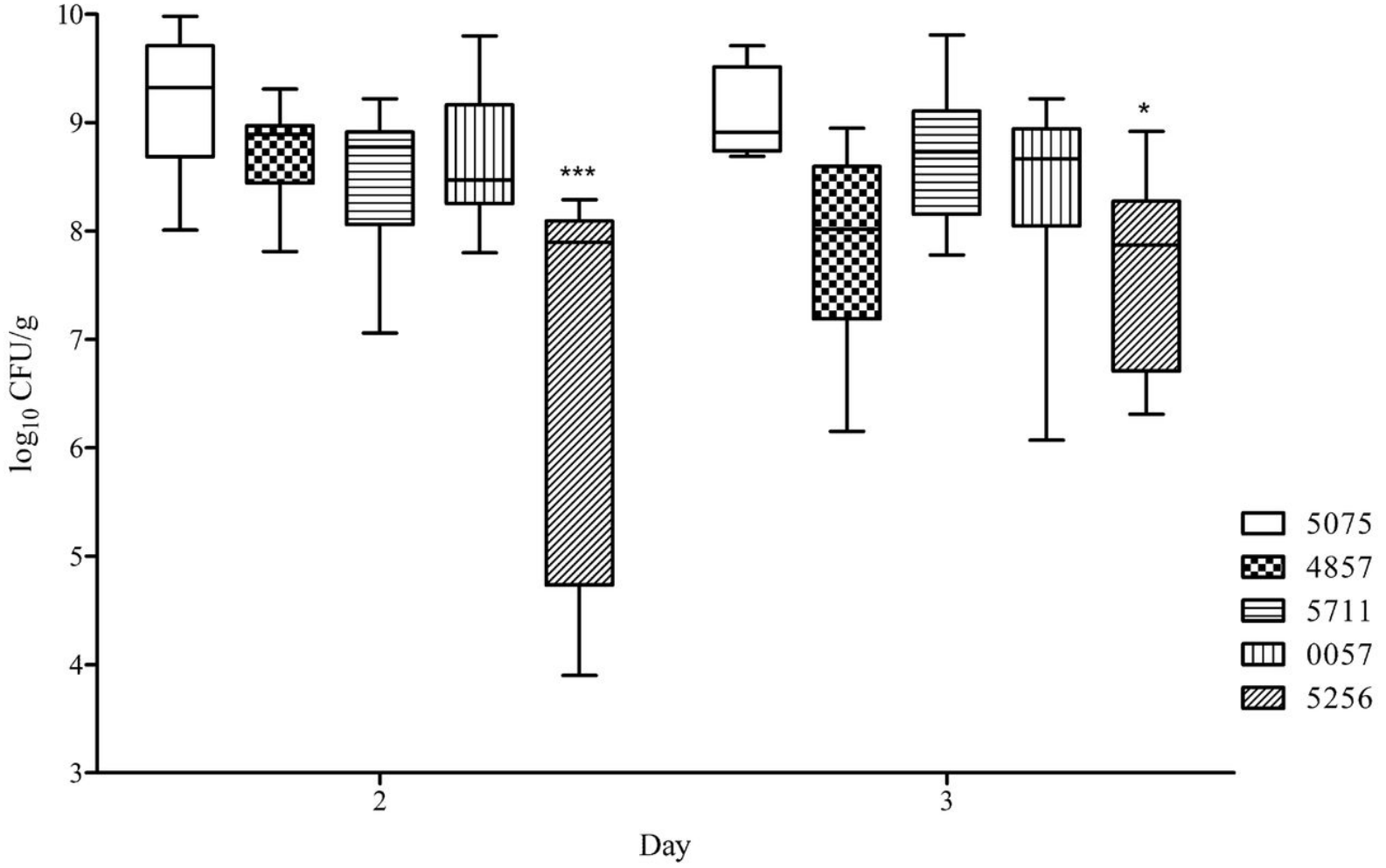


Murine Pulmonary Model

Intranasal inoculation

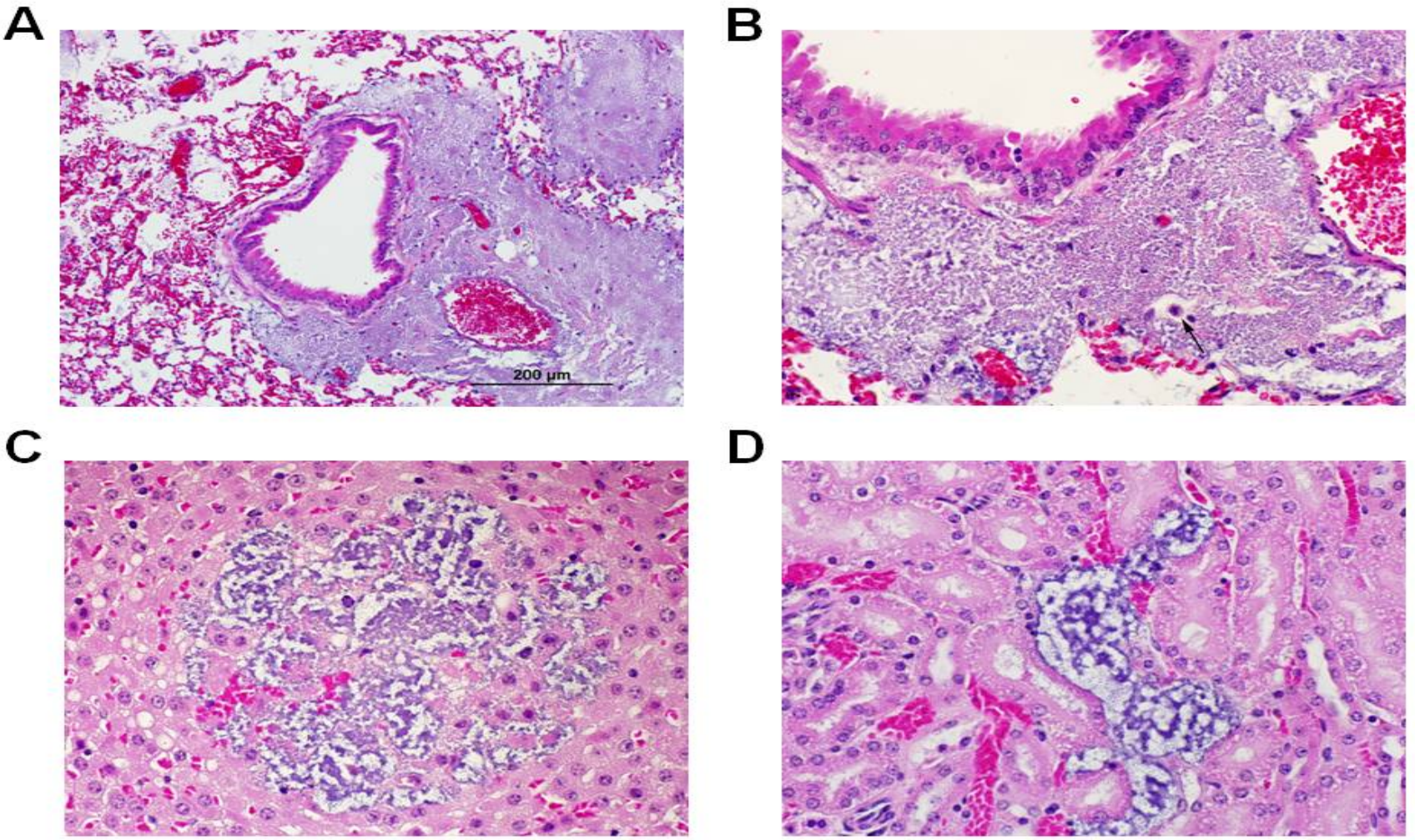


Bacterial levels in lung tissue



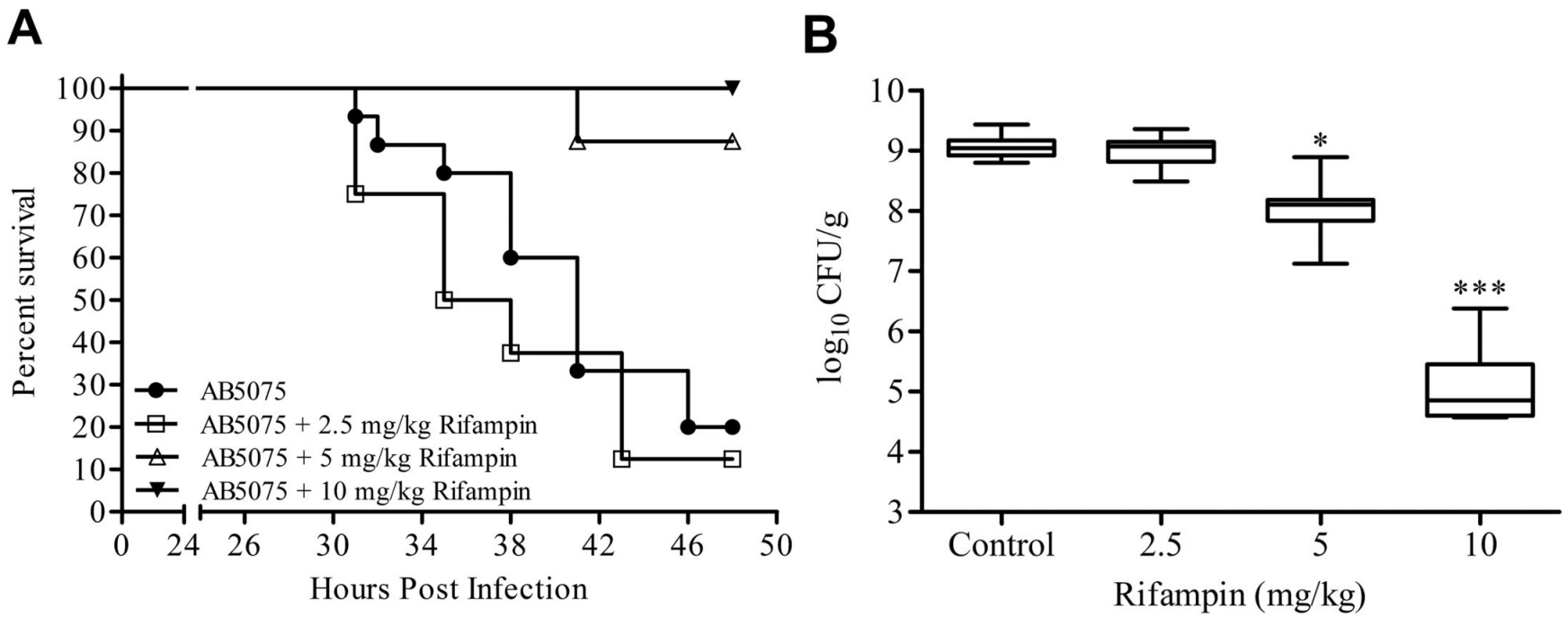
Anna C. Jacobs et al. mBio 2014; doi:10.1128/mBio.01076-14

Histopathology



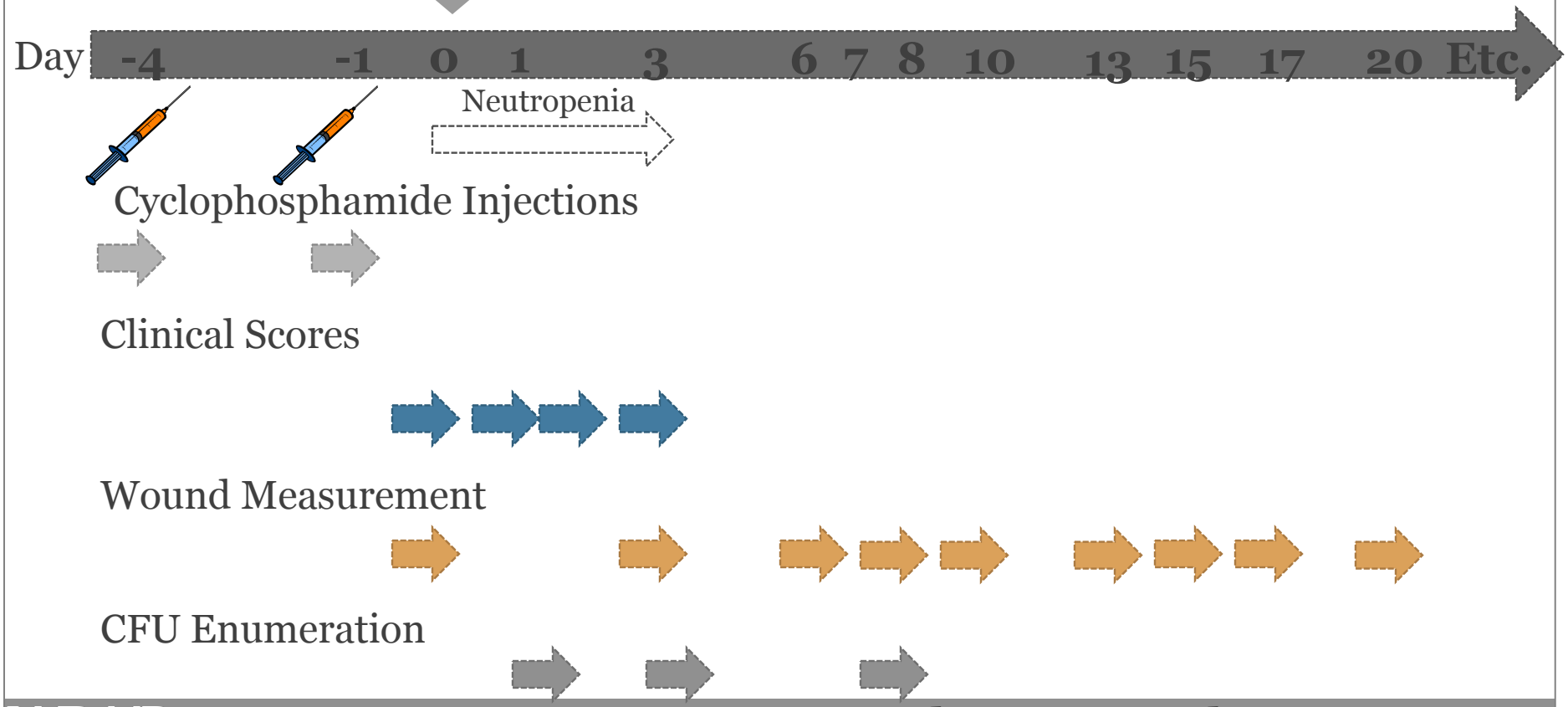
Hobson et al. 2016

Rifampin as proof of concept



Anna C. Jacobs et al. mBio 2014; doi:10.1128/mBio.01076-14

Mouse Wound Model

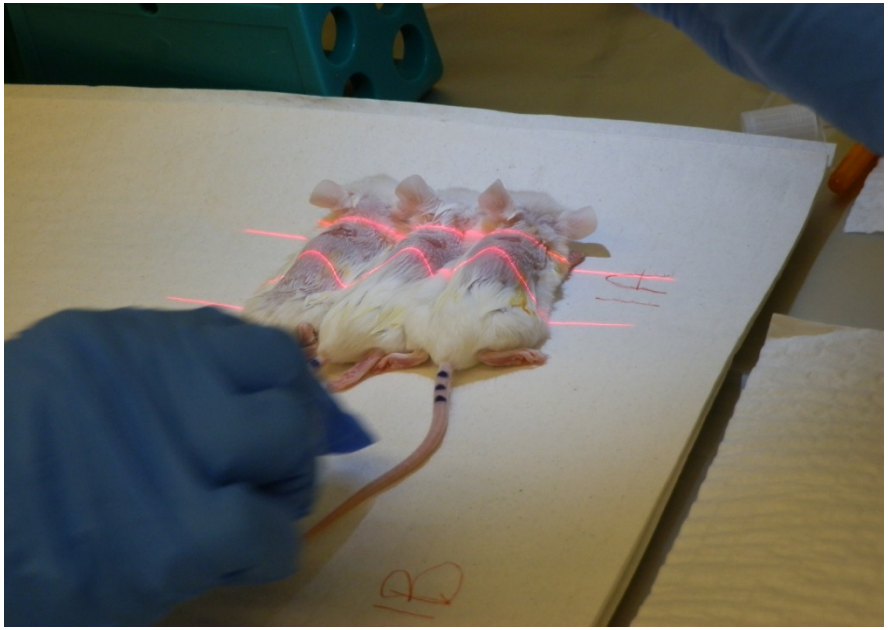


Surgical Procedure

Anesthetized and Shaved

6 mm punch “biopsy” wound

Wound Measurement



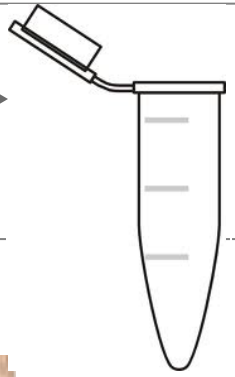
Inoculation and Dressing

Bacteria inoculation – 25 µl volume
5.0×10^4 CFU

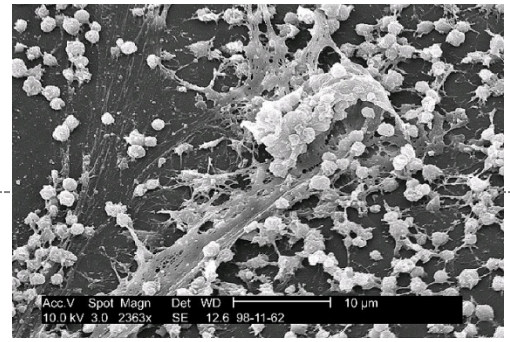
Placement of Tegaderm dressing over wound

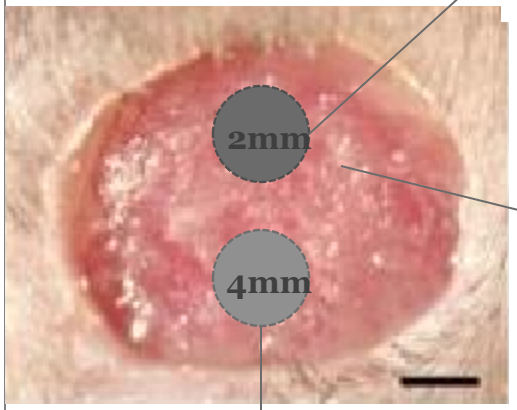
Removed Tegaderm

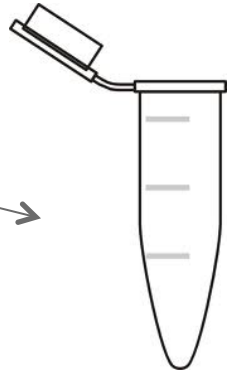
Place in 1 mL PBS



SEM/CSLM







Flash freeze in dry ice/methanol bath

Preserved for 16S FLX

Place in 1mL RNAProtect

Place directly in 1.5mL Eppie

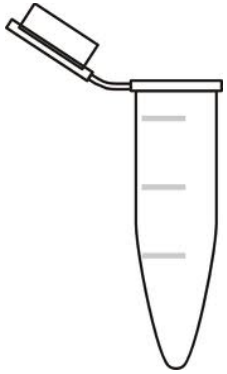
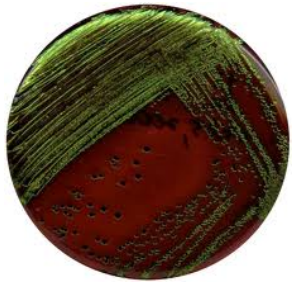


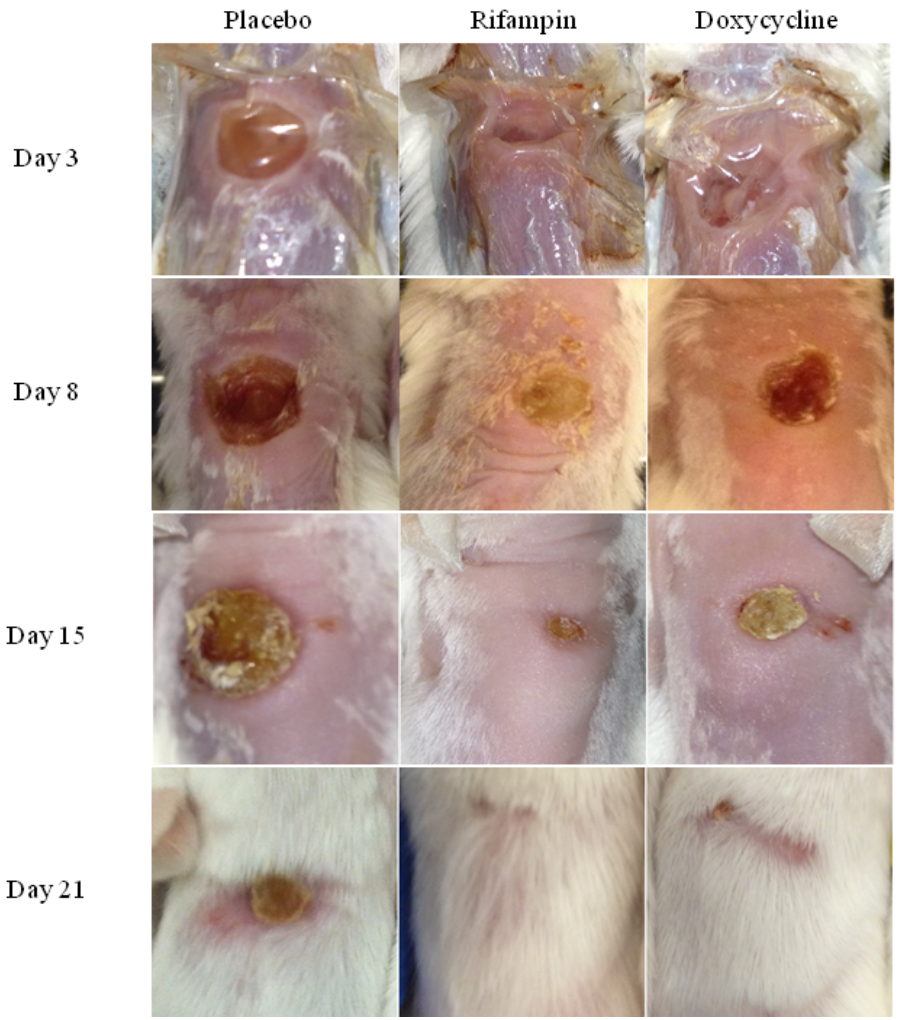
Plate EMB Agar Gram (-)



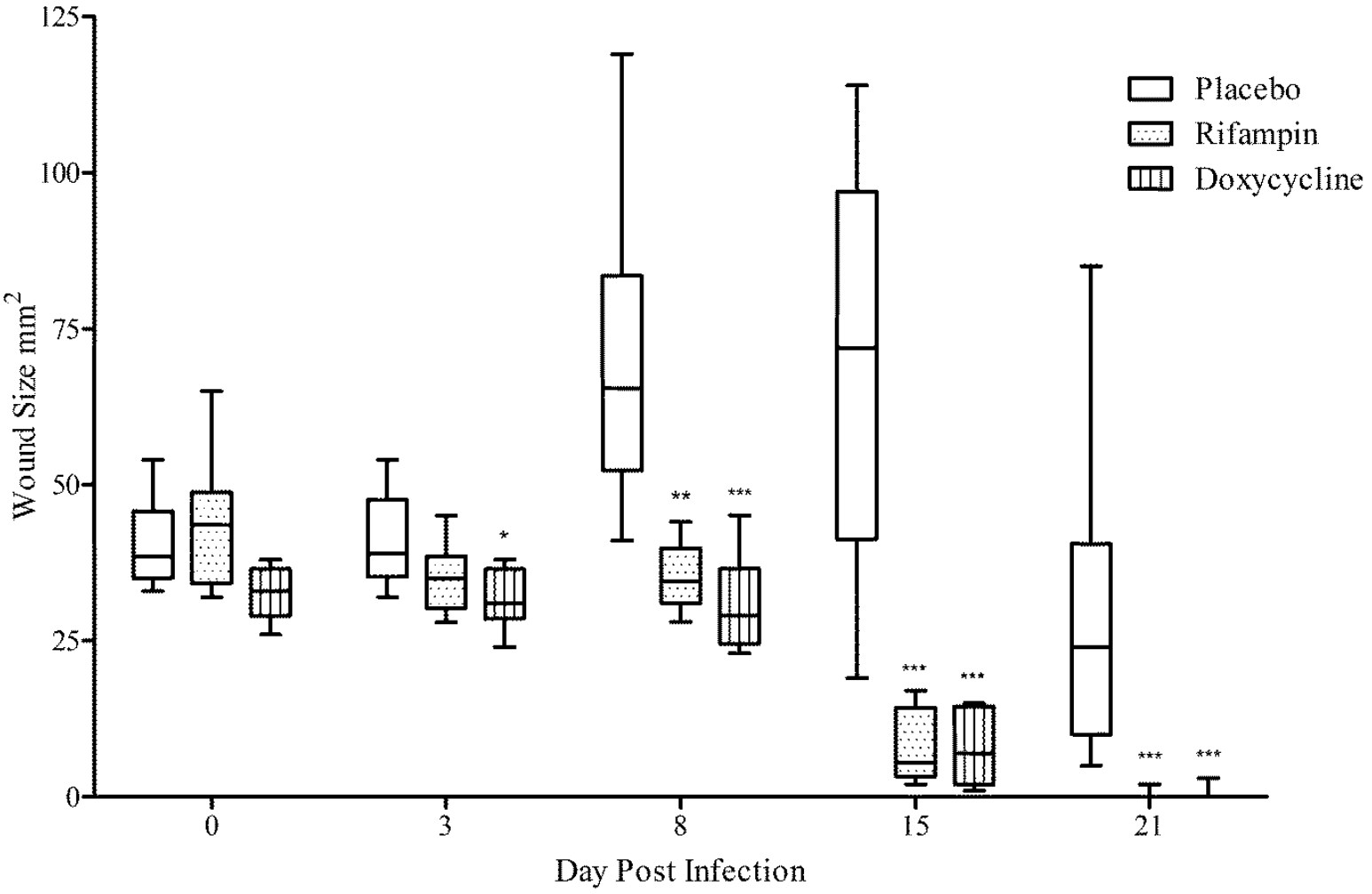
Homegenize and Dilute

Plate Mannitol Salt Agar Gram (+)

Model Validation – Gross pathology

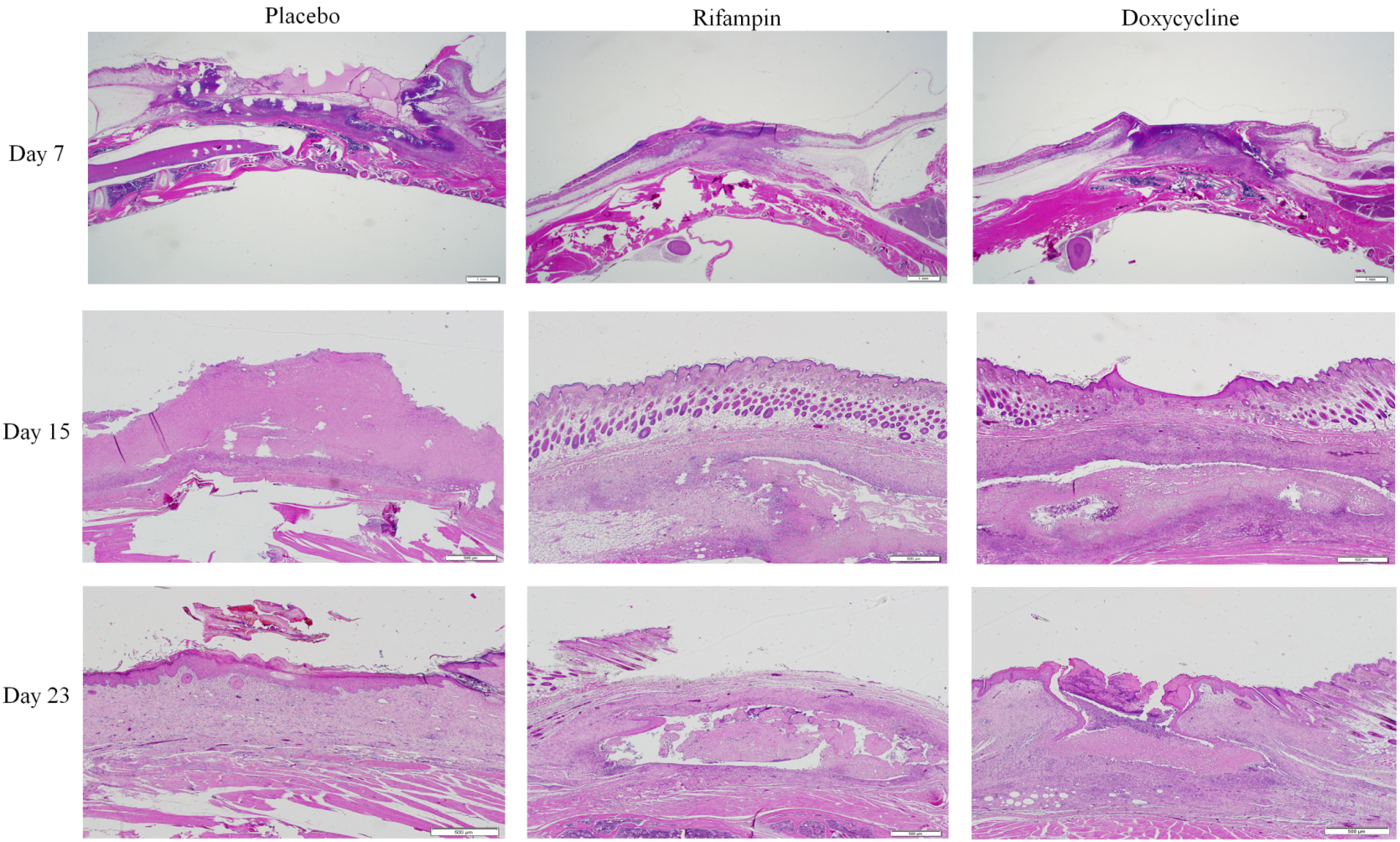


Wound area over time

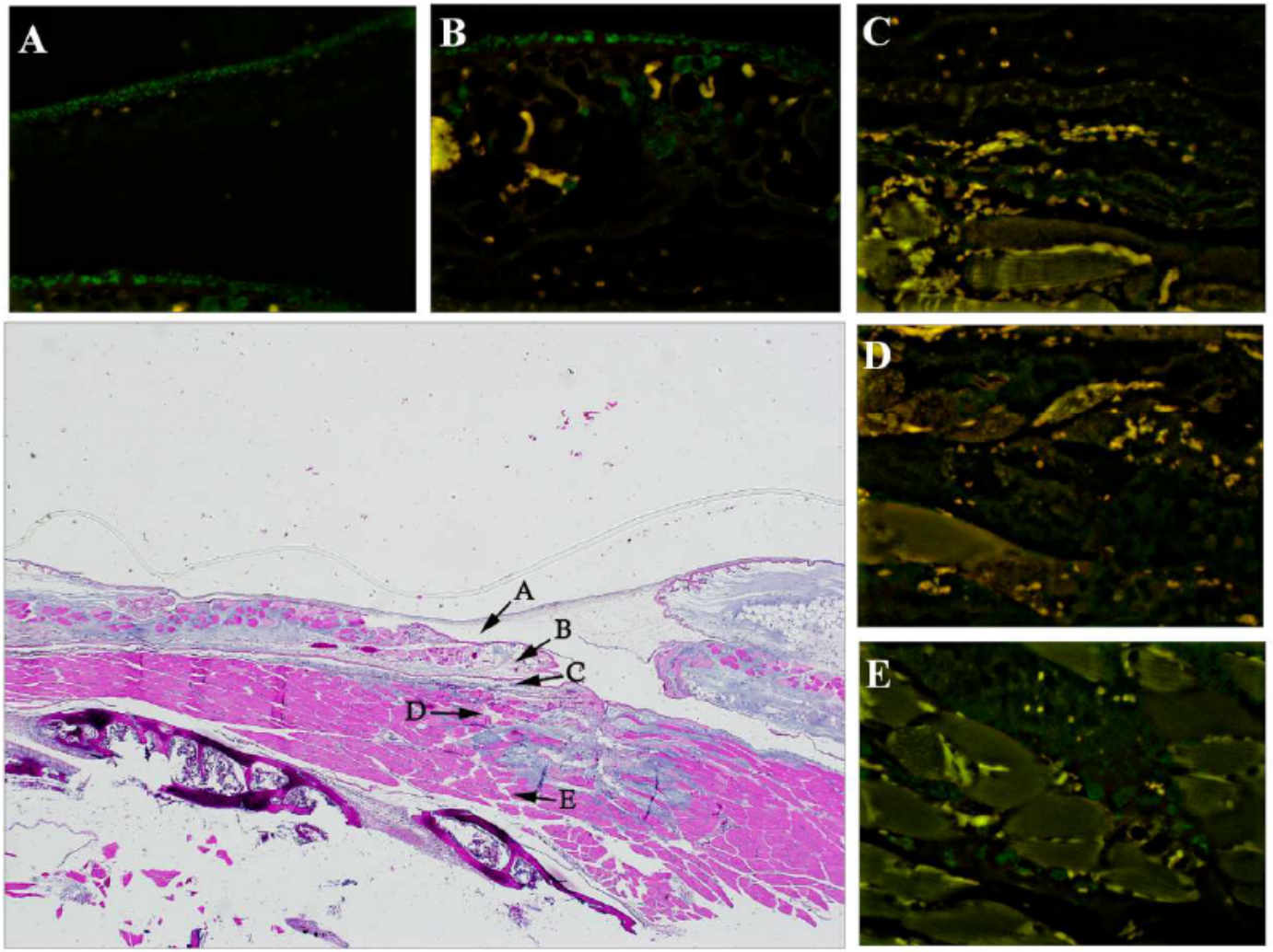


Thompson et al. *AAC*, 2014

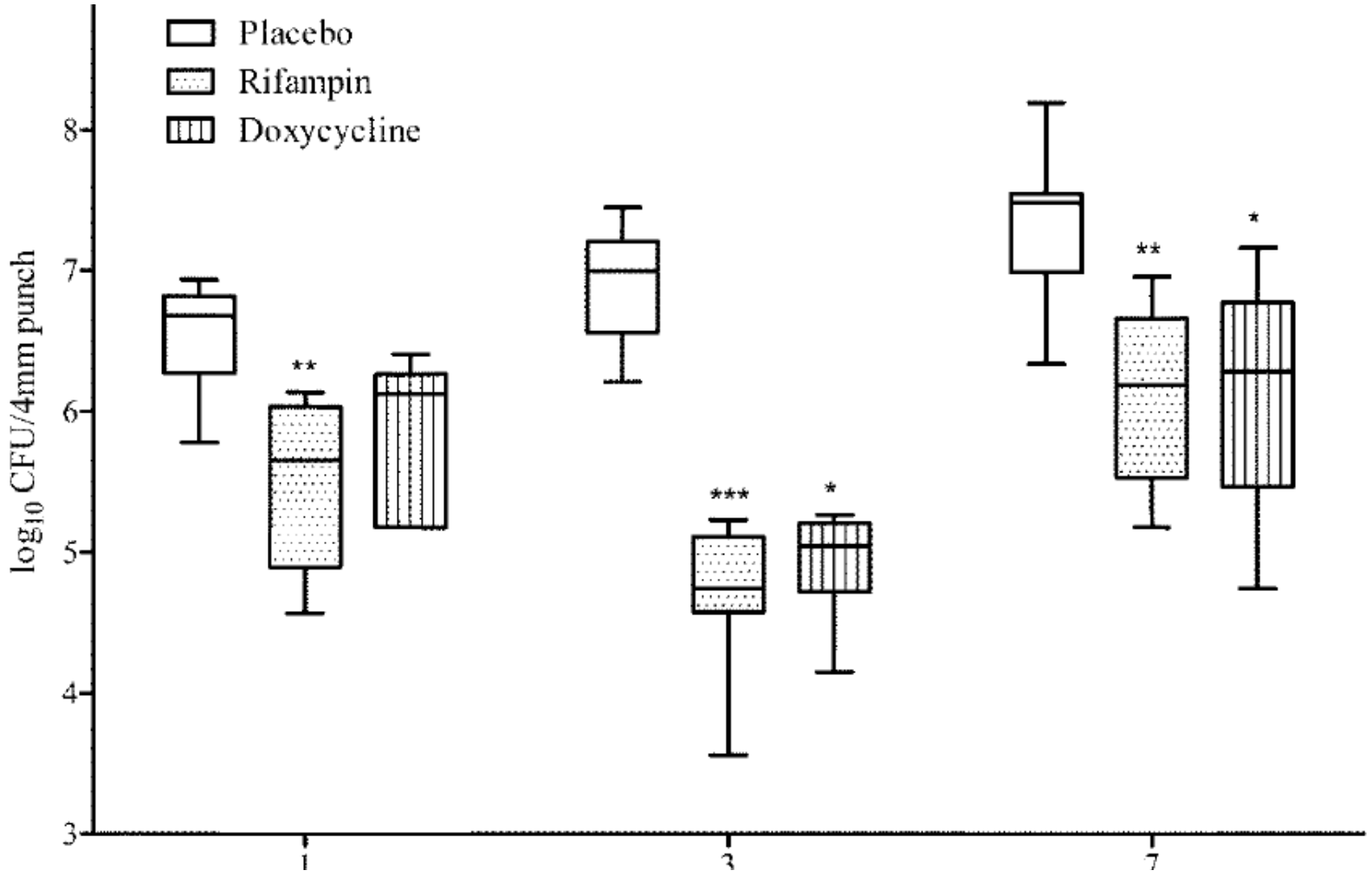
Histopathology



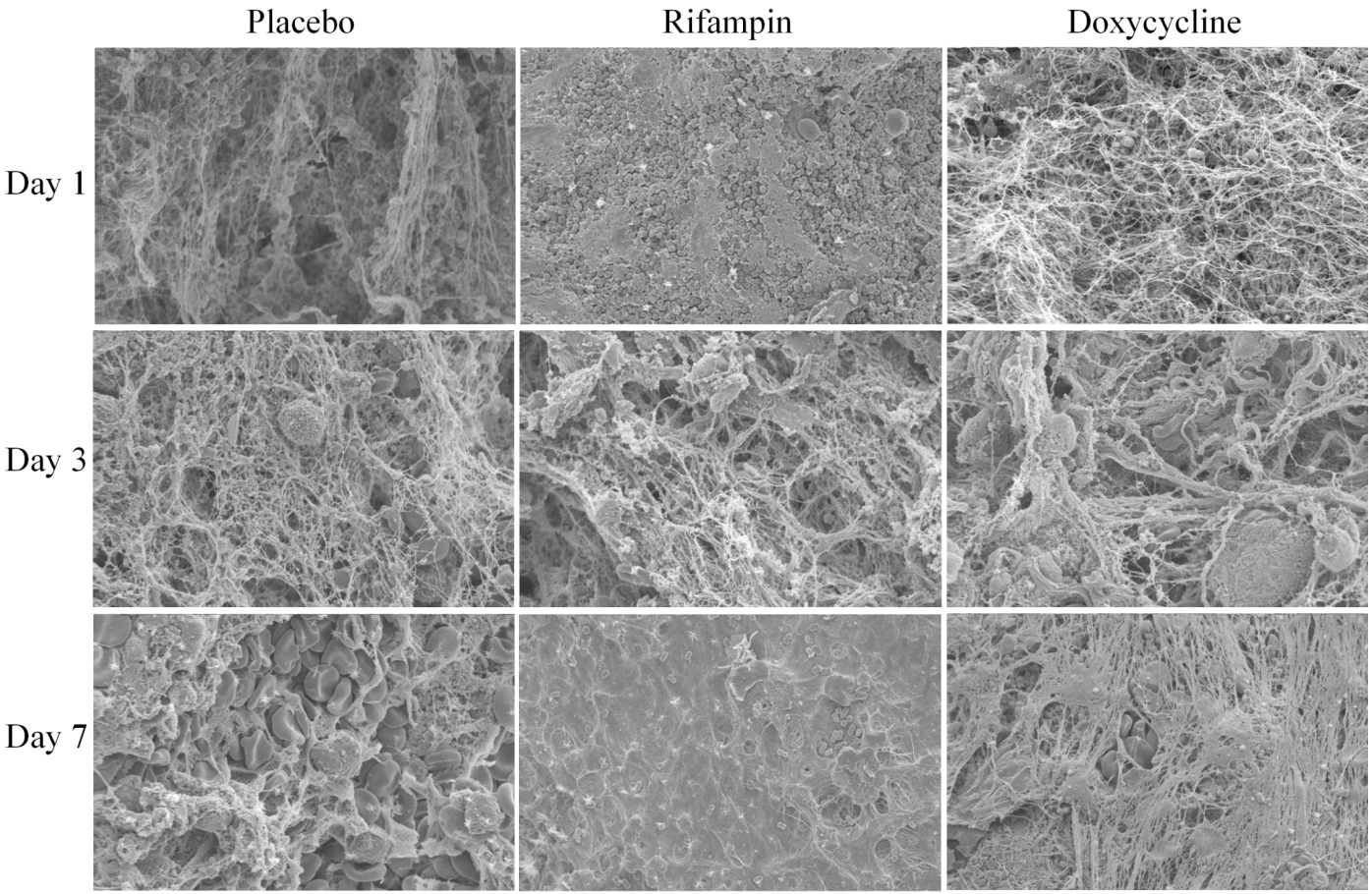
PNA FISH - 16S Probe - AdvanDx



CFU/g wound tissue



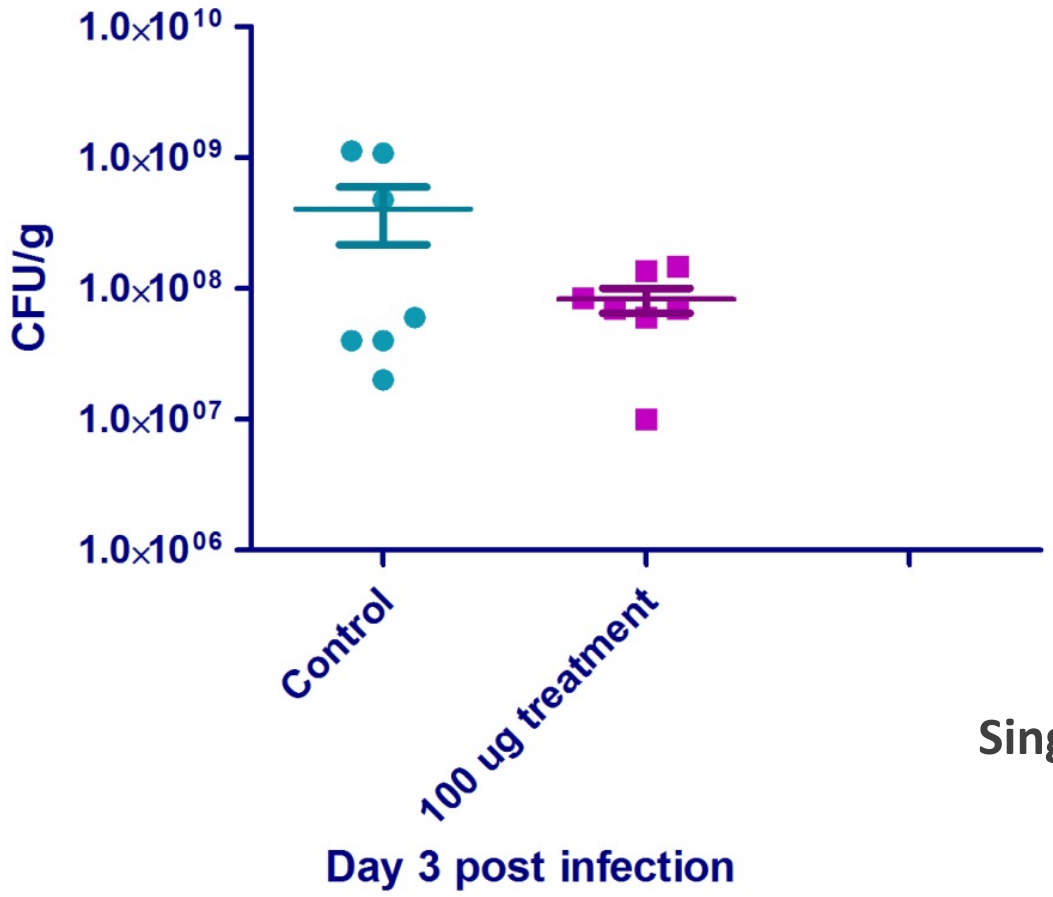
Biofilms – *in vivo*



mAb Testing
αHcp in the murine wound model of infection

Singh *et al*. unpublished

mAb Testing
In vivo - CFU/g Wound Tissue



Singh *et al*. unpublished

Endpoints – Mouse Wound

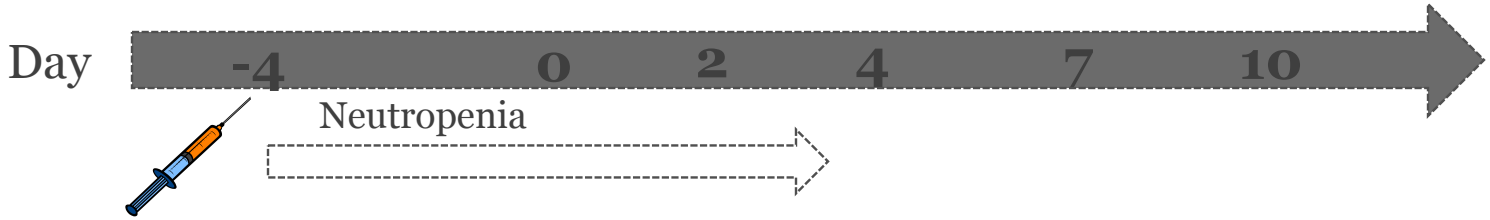
- Time to close – wound area over time
- CFU/g – tissue
- Biofilm evaluation – 16S probe and SEM
- Gross pathology
- Histopathology
- Cytokine/Chemokine
- Microbiome evaluation
- Animal Weight

Further optimization – Mouse Wound

- Addition of uranyl nitrate – 5 mg/kg – humanize excretion
- Other strains of *A. baumannii*
- Wound < dissemination < sepsis
- Other mouse strains
  - Diabetic mice
  - A/J mice – delayed neutrophil response
  - C3HeB/FeJC3HeB/FeJ
  - Humanized mice (DRAG)

- Immune response

Pig Wound Model



Cyclophosphamide lowered to 25 mg/kg and given only once

CFU Enumeration

Histopathology/Immunohistochemistry/SEM

Complete Blood Count

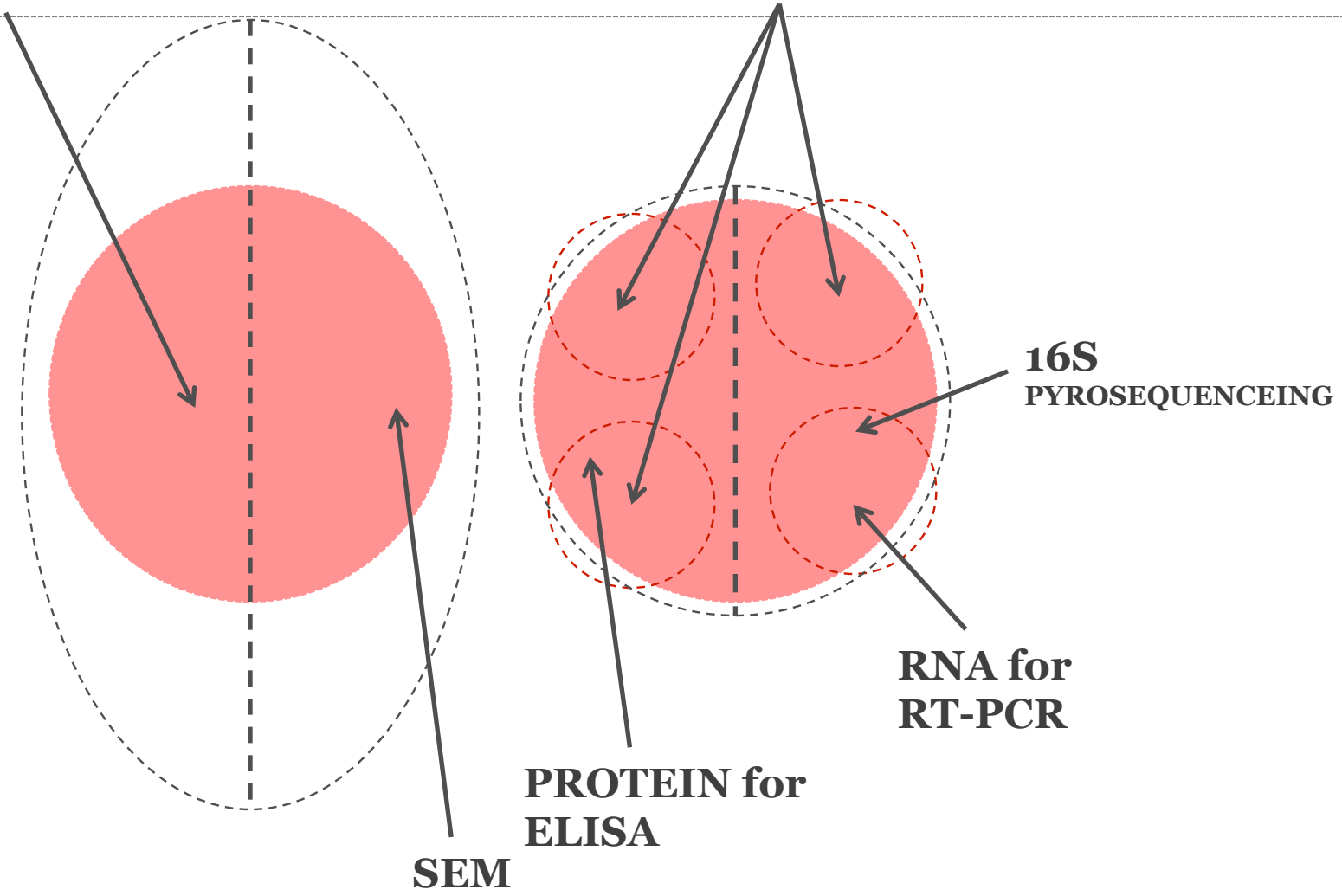
Weight, Clinical Signs

Host Response: ELISA and RT-PCR

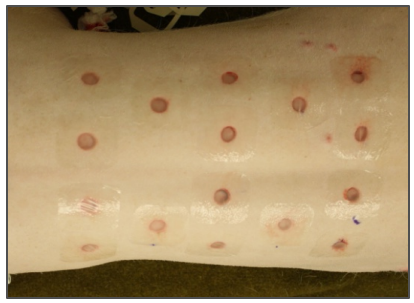
Pathogen Characterization: 16S and RT-PCR

Black et al. unpublished

HISTOPATHOLOGY and IMMUNOHISTOCHEMISTRY



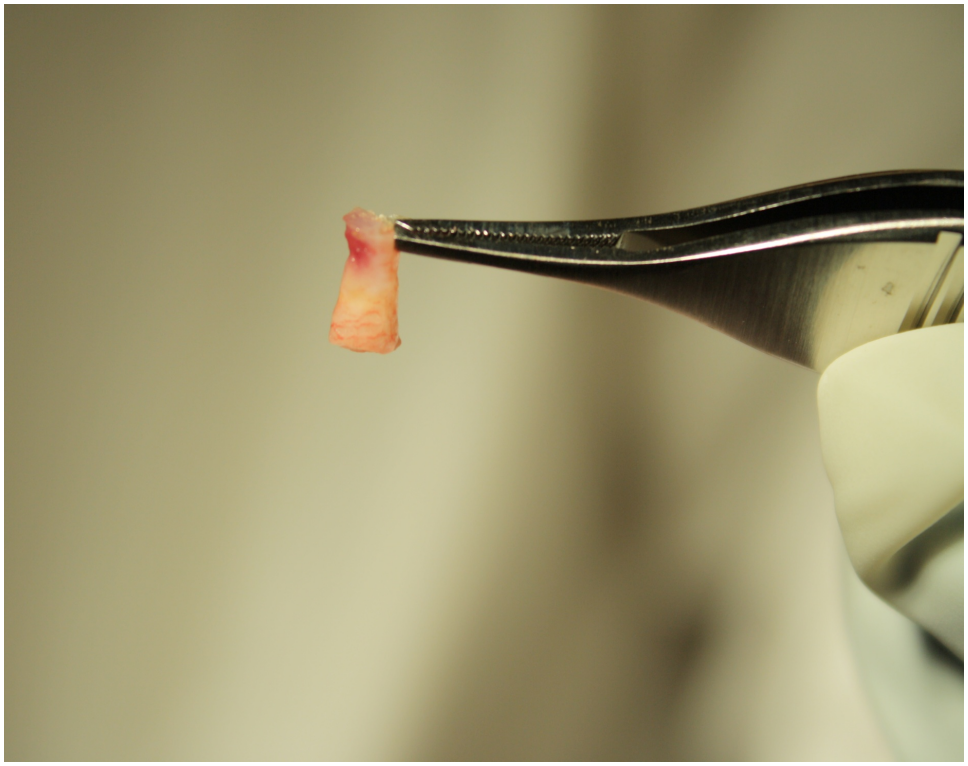
Wound Layout

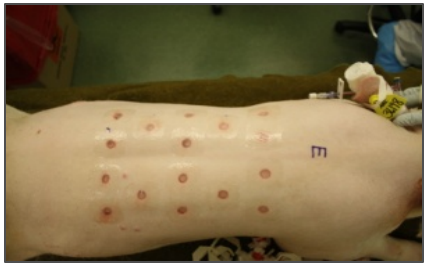


RANDOMIZED

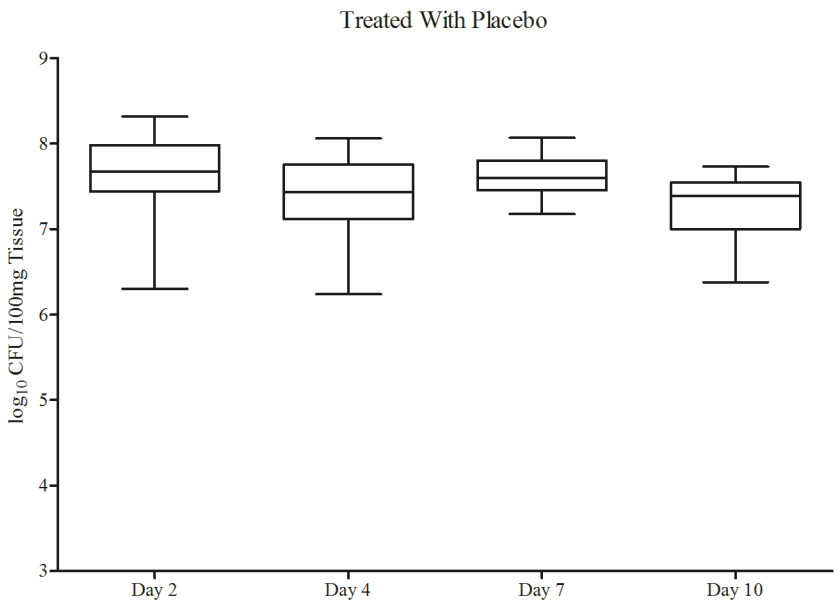
HISTOPATHOLOGY and IMMUNOHISTOCHEMISTRY

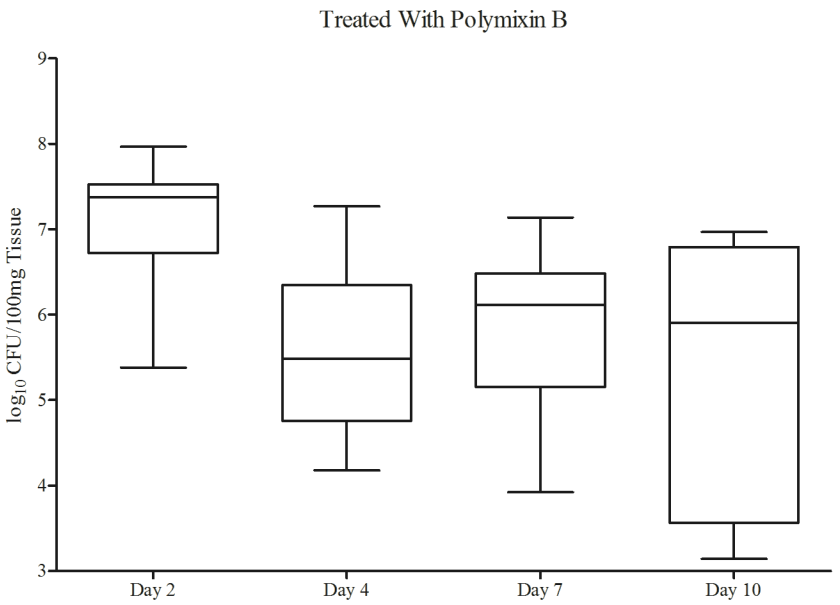
Pictures of aspects of model





CFU reduction over time





Black et al. unpublished

Summary

- *Acinetobacter baumannii* can be an aggressive pathogen in some compromised hosts.
- We have developed two murine models that rely on neutropenia that we feel reflect the appropriate pathogenesis and can be used to test novel antibacterial approaches.
- We have developed a porcine model using similar techniques.
- On the whole, we have tested over 14 different antibacterials using our mouse models and compared them to standard of care.

Project	Outcome
Predatory Bacteria	Positive – minor effect – still in progress
Bacteriophage	Positive – paper published/strain-dependent
Monoclonal Antibody	Positive – 40% survival - still in progress
[redacted]	Negative – compounds too hydrophobic
Gallium [redacted]	Positive – minor effect – paper published
Gallium-[redacted]	Positive – still in progress
Hyperbaric Oxygen Therapy (HBOT)	Negative
CARB	In progress
[redacted] Peptide	Negative – not stable
Probiotic ([redacted])	Negative – still in progress
Iron Chelator	Negative – chelator/bacterial species dependent
Copper Resistance	Positive – paper published/follow-up in prep
[redacted] *A. baumannii*	Positive – paper published/follow-up in prep
Colistin Resistance	Positive – paper published
A. baumannii hyper-virulent	Positive – paper published
[redacted]	Positive – paper in prep
[redacted] Inhibition	Negative

Acknowledgements

Bacterial Diseases Branch
LTC Stuart D. Tyner, Ph.D.

Wound Infections Dept.
MAJ Samantha Demons, Ph.D.
MAJ Jonathan Shearer, DVM
Janos Luka, Ph.D.
Vlado Antonic, Ph.D.
Anna Jacobs, Ph.D.
Schroeder Noble, Ph.D.
Ryan Reddinger, Ph.D.
Alexandria Reinhart, Ph.D.
Iswar Soojhawon, Ph.D.
Yuanzheng Si
Rae Heitkamp
Melin Kanasseril
Shweta Singh
Yonas Alamneh
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Fialka Ward

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Defeating combat wound infections