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

THE BAD ACTORS – ESKAPEE

• *Enterobacter cloacae*
• *Staphylococcus aureus*
• *Klebsiella pneumoniae*
• *Acinetobacter baumannii*
• *Pseudomonas aeruginosa*
• *Enterococcus faceium*
• *Escherichia coli*

Wounded Numbers in OIF + OEF +OND +OIR +OFS (2016)

<p>| | |</p>
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<td>WIA</td>
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Sources: DCAS website Fisher et al. 2015

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

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Acinetobacter baumannii strains - PFGE

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

Unpublished data
Motility and Optical Mapping Diversity

McQueary et al. 2012
Virulence in *Galleria mellonella*

Jacobs et al., *mBio* 2014

\[ N = 20 \]

\[ P \text{ value} = .0001 \]

Mantel – Cox Test
Murine Pulmonary Model

Intranasal inoculation

Day
-4 -1 0 1 3 6 10

Cyclophosphamide Injections
Neutropenia

Clinical Scores
CFU Enumeration

Jacobs et al., mBio 2014
Bacterial levels in lung tissue

Day

log_{10} CFU/g

5075
4857
5711
0057
5256
Histopathology

A

B

C

D

Hobson et al. 2016
Rifampin as proof of concept

[Graph A: Percent survival over time for different doses of Rifampin.]

[Graph B: Log10 CFU/g vs Rifampin dosage.]

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

Day -4 -1 0 1 3 6 7 8 10 13 15 17 20 Etc.

- Cyclophosphamide Injections
- Neutropenia
- Clinical Scores
- Wound Measurement
- CFU Enumeration

Thompson et al. AAC, 2014
Surgical Procedure

Anesthetized and Shaved

6 mm punch “biopsy” wound

Thompson et al., 2014
Wound Measurement
Inoculation and Dressing

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

Placement of Tegaderm dressing over wound

Thompson et al., 2014
Removed Tegaderm

Place in 1 mL PBS

Place in 1 mL RNAProtect

Flash freeze in dry ice/methanol bath

Preserved for 16S FLX

2mm

4mm

Place directly in 1.5mL Eppie

Plate EMB Agar Gram (-)

Plate Mannitol Salt Agar Gram (+)

Homegenize and Dilute

SEM/CSLM

Thompson et al., 2014
Model Validation – Gross pathology

Day 3
Placebo  Rifampin  Doxycycline

Day 8

Day 15

Day 21
Wound area over time

Day Post Infection

Wound Size mm²

Placebo
Rifampin
Doxycycline
Histopathology

Day 7
Placebo
Rifampin
Doxycycline

Day 15

Day 23

Thompson et al. AAC, 2014
PNA FISH - 16S Probe - AdvanDx

Thompson et al. AAC, 2014
CFU/g wound tissue

- Placebo
- Rifampin
- Doxycycline

Log_{10} CFU/4mm punch

Thompson et al. AAC, 2014
Biofilms – *in vivo*

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<tr>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
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<td>Placebo</td>
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<td>Doxycycline</td>
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</table>
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/FeJ/C3HeB/FeJ
  - Humanized mice (DRAG)

- Immune response
Pig Wound Model

Cyclophosphamide lowered to 25 mg/kg and given only once

- Neutropenia

**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

SEM

CFU

RNA for RT-PCR

PROTEIN for ELISA

16S PYROSEQUENCING

Black et al. unpublished
Wound Layout

RANDOMIZED HISTOPATHOLOGY and IMMUNOHISTOCHEMISTRY

Black et al. unpublished
Pictures of aspects of model

Black et al. unpublished
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.
<table>
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<th>Project</th>
<th>Outcome</th>
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<tr>
<td>Predatory Bacteria</td>
<td>Positive – minor effect – still in progress</td>
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<tr>
<td>Bacteriophage</td>
<td>Positive – paper published/strain-dependent</td>
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<td>Monoclonal Antibody</td>
<td>Positive – 40% survival - still in progress</td>
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<tr>
<td>Gallium</td>
<td>Negative – compounds too hydrophobic</td>
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<tr>
<td>Gallium</td>
<td>Positive – minor effect – paper published</td>
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<td>Hyperbaric Oxygen Therapy (HBOT)</td>
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<tr>
<td>CARB</td>
<td>Negative</td>
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<td>Peptide</td>
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<td>Probiotic</td>
<td>Negative – not stable</td>
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<tr>
<td>Probiotic</td>
<td>Negative – still in progress</td>
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<tr>
<td>Iron Chelator</td>
<td>Negative – chelator/bacterial species dependent</td>
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<tr>
<td>Copper Resistance</td>
<td>Positive – paper published/ follow-up in prep</td>
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<tr>
<td>A. baumannii</td>
<td>Positive – paper published/ follow-up in prep</td>
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<td>Colistin Resistance</td>
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Acknowledgements

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