Challenges of *in vivo* studies to support pre-clinical to clinical translation

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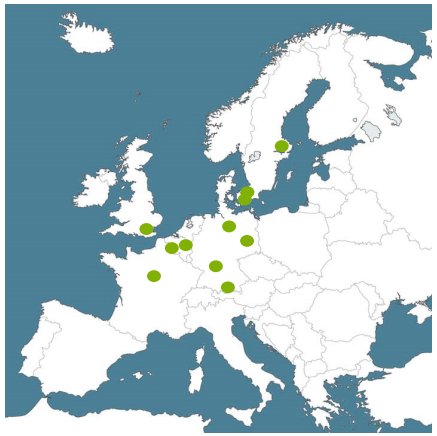
On behalf of the COMBINE Consortium

AMR Accelerator: Public-Private collaboration with the shared goal of progressing the development of new medicines to treat or prevent resistant bacterial infections (www.amr-accelerator.eu)

COMBINE:
Collaboration for prevention and treatment of MDR bacterial infections

Scientific goals:

- **optimise and standardise animal infection models to advance translation of non-clinical efficacy data to clinical trial outcomes**
- improve statistical and pharmacometric analyses of clinical data
- develop optimized clinical trial designs



Universities, research organisations, public bodies, non-profit groups:

- Uppsala University (UU) Sweden Coordinator
- Paul-Ehrilch-Institut (PEI) Germany
- Fraunhofer Gesellschaft (FRAUNHOFER) Germany
- Statens Serum Institut (SSI) Denmark
- BEAM Alliance (BA) France

SMEs:

- Asclepia (AC) Belgium
- GRIT42 (G42) Denmark
- BIOCOM (BC) Germany

EFPIA companies:

- GSK United Kingdom Project Lead
- Evotec (EVT) Germany
- Janssen (JNJ) Belgium

https://amr-accelerator.eu/project/combine

Improve understanding of animal infection model reproducibility and translation to clinical efficacy

Problem:

- Animal infection models are excellent tools, yet translational gaps remain
- Methods used for study conduct & analyses impact results
- Lack of standardization hinders interpretation & comparison

Goals:

- Develop standardised animal infection model protocol
- Benchmark standard model using relevant control compounds
- Establish in vivo reference strain bank supported by data from the model
- Provide framework for PK/PD analysis & mathematical modelling
- Improve understanding of preclinical-to-clinical translation

Three main activities

Validate a standardized infection model

- Select what model to standardize
- Generate efficacy data for control antibiotics using candidate strains

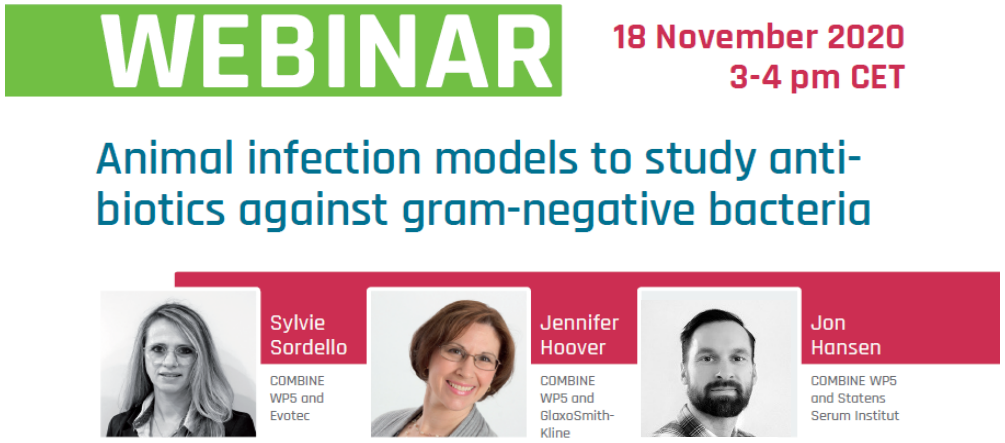
Establish Reference Strain Bank

- Identify strains that can be made available to the AMR community
- Select candidate strains that perform well in a standard model across labs

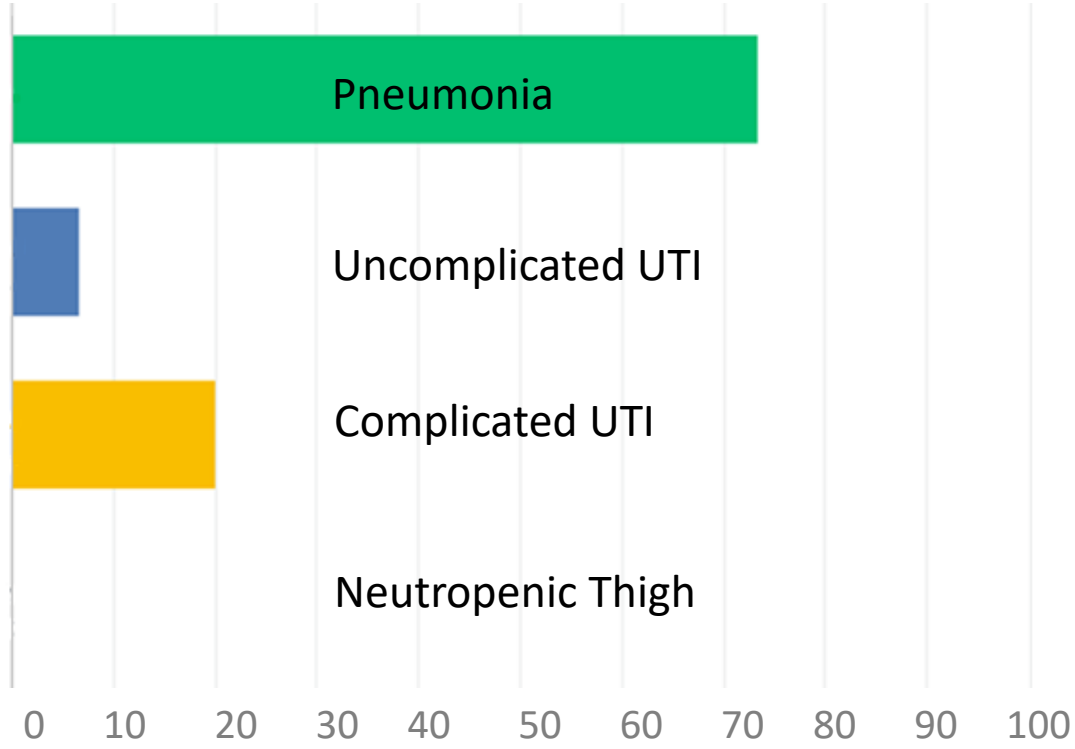
Improve Preclinical-to-Clinical Translation

- Demonstrate how to best interpret and use the data for PK/PD modeling
- Investigate how response in our standard model translates to the clinic

Selection of murine infection model to standardize

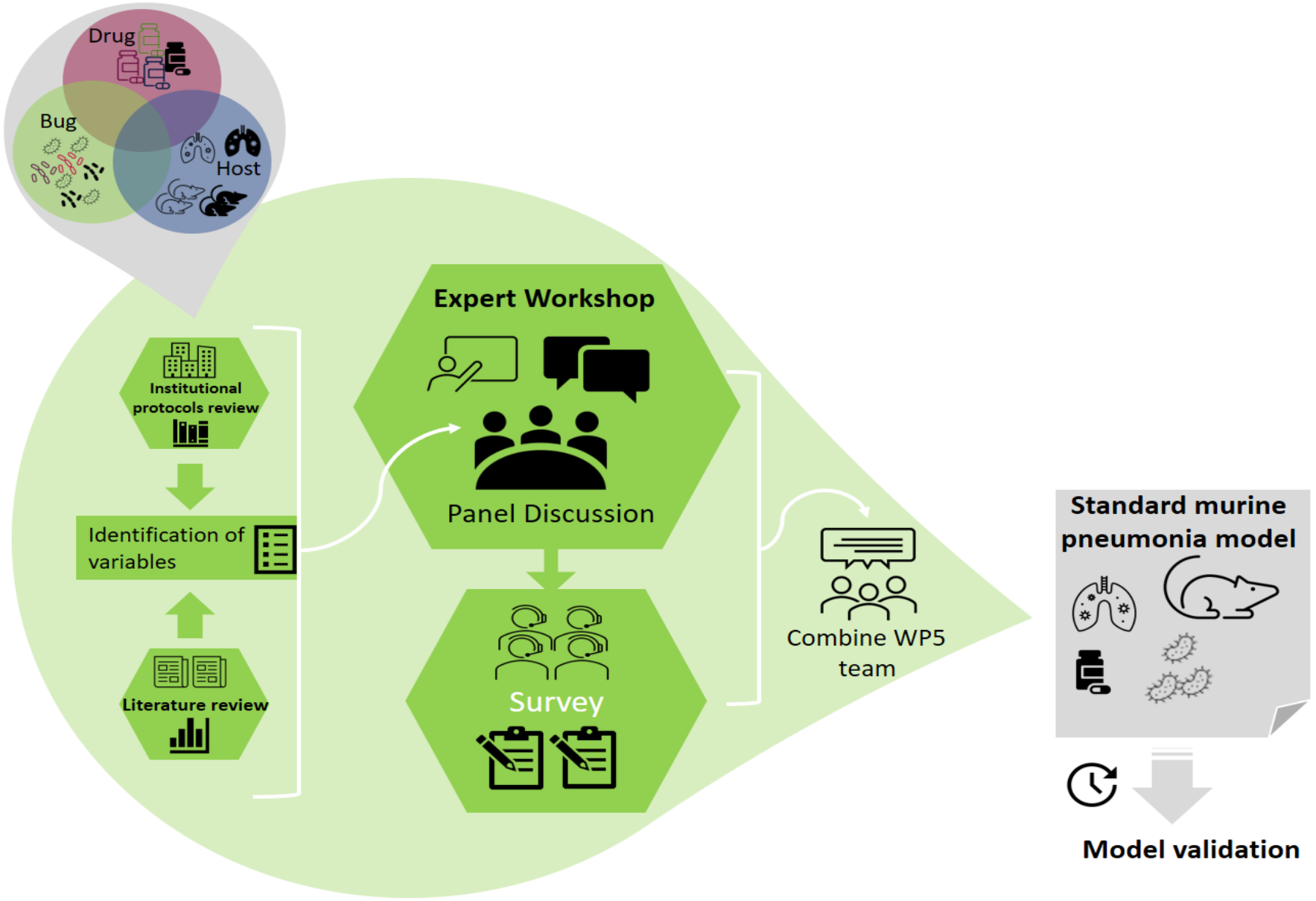


- Presentations on models to study antibiotics vs. Gram-negative pathogens
- Survey among participants to identify greatest need for model improvement



Model with greatest translational gap (% of survey participants)

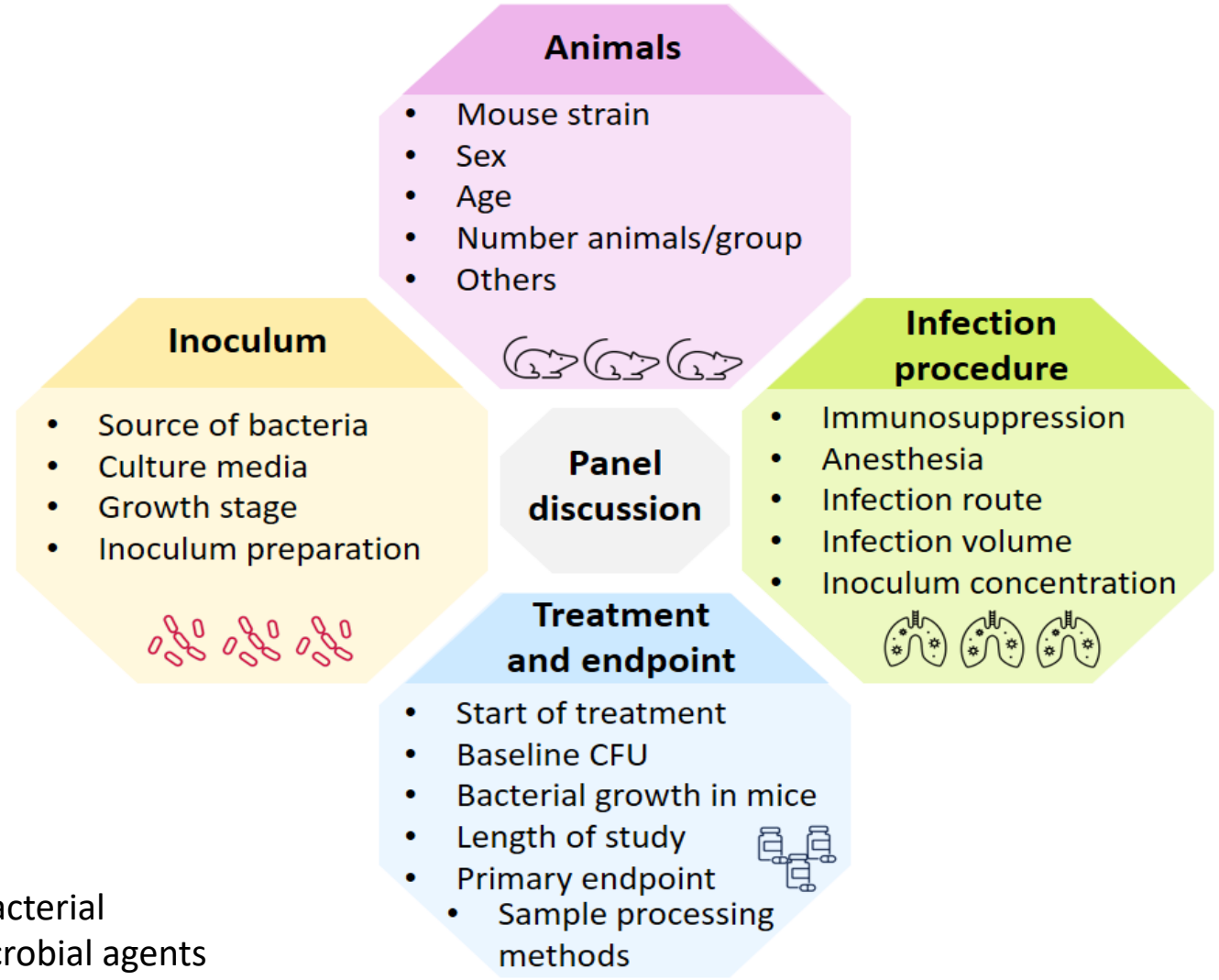
Standardization Process



Assessment of methods for acute pneumonia models with *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii*

- Literature Review
- Review of institutional protocols

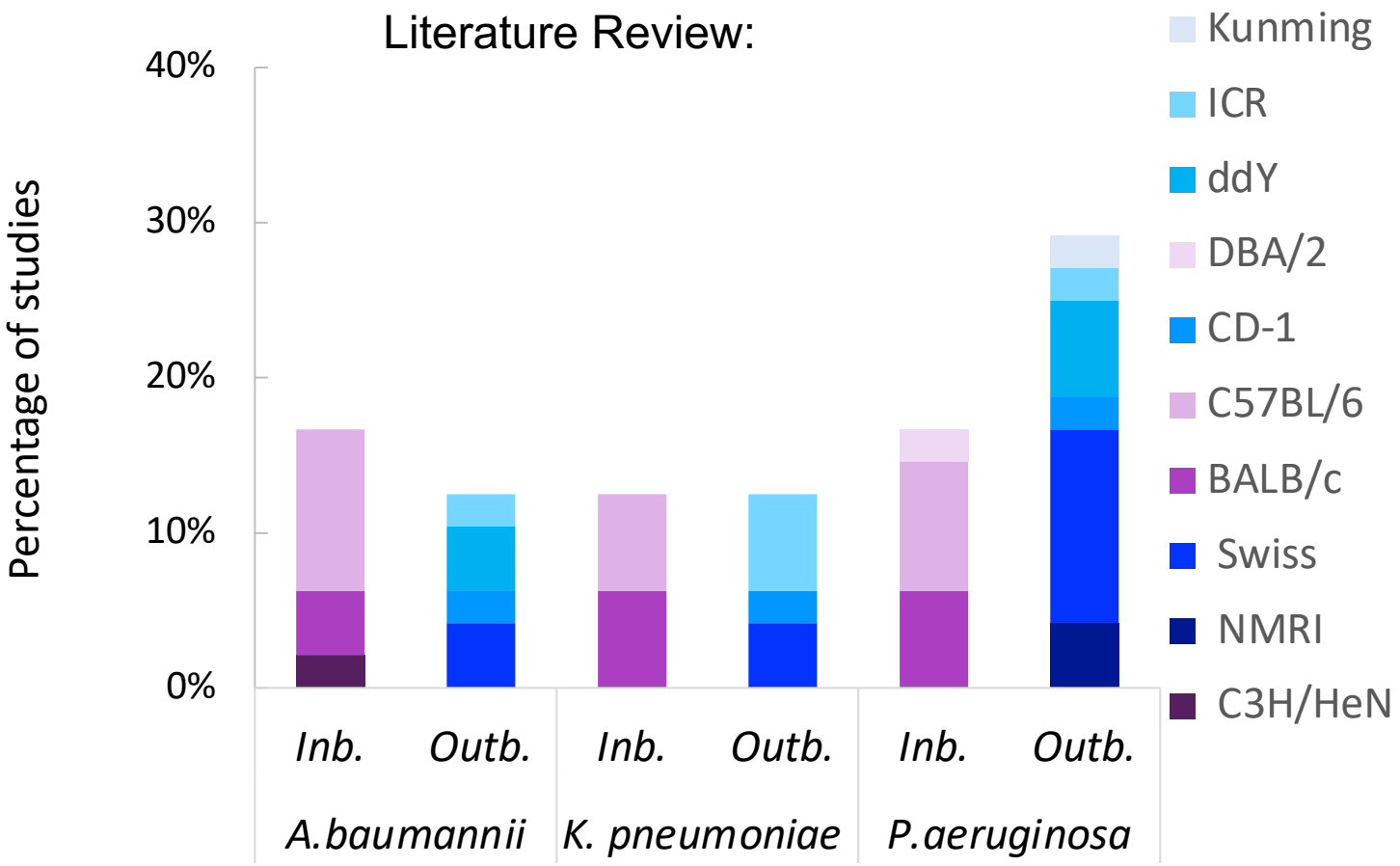
Substantial differences in study methods



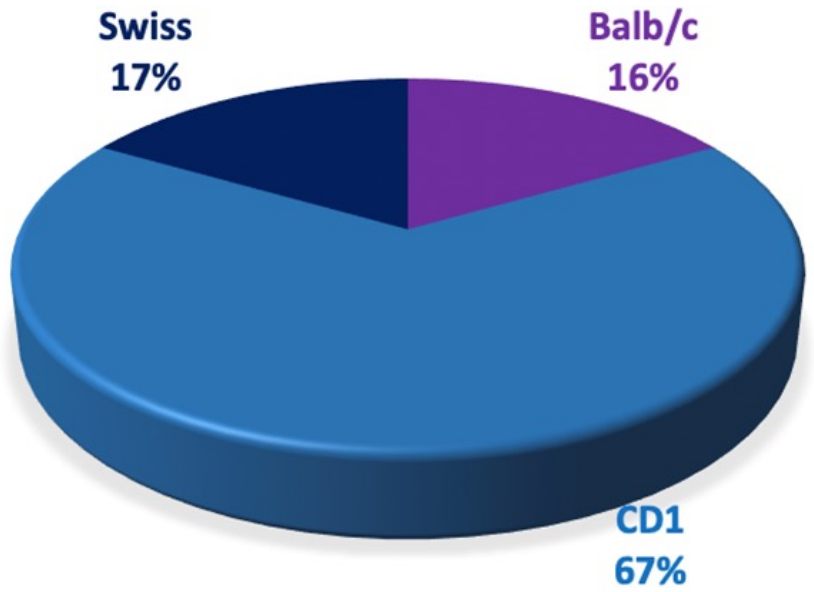
Rakel Arrazuria et.al. Variability of murine bacterial pneumonia models used to evaluate antimicrobial agents

Identification of key variables: Mice

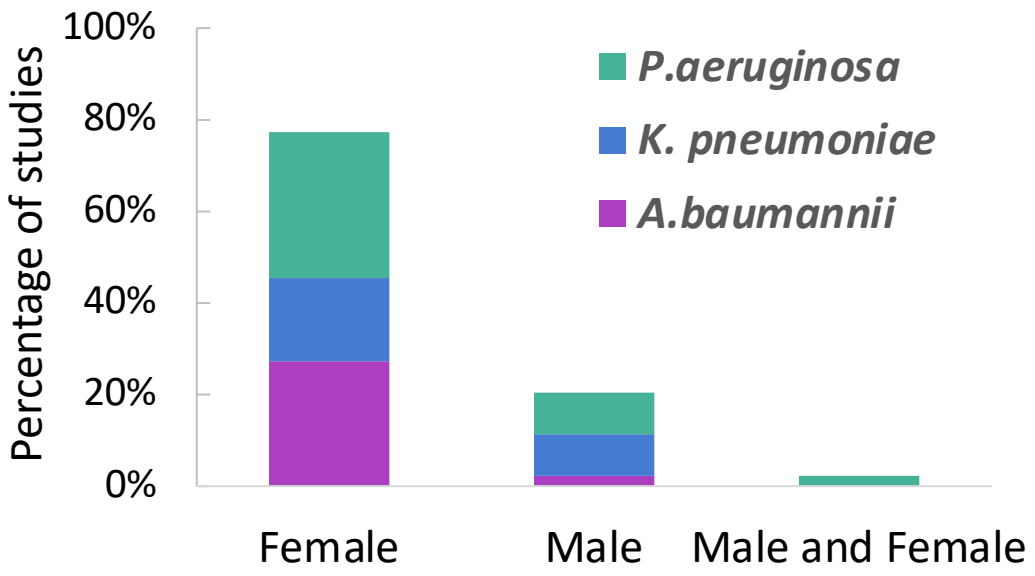
Literature Review:

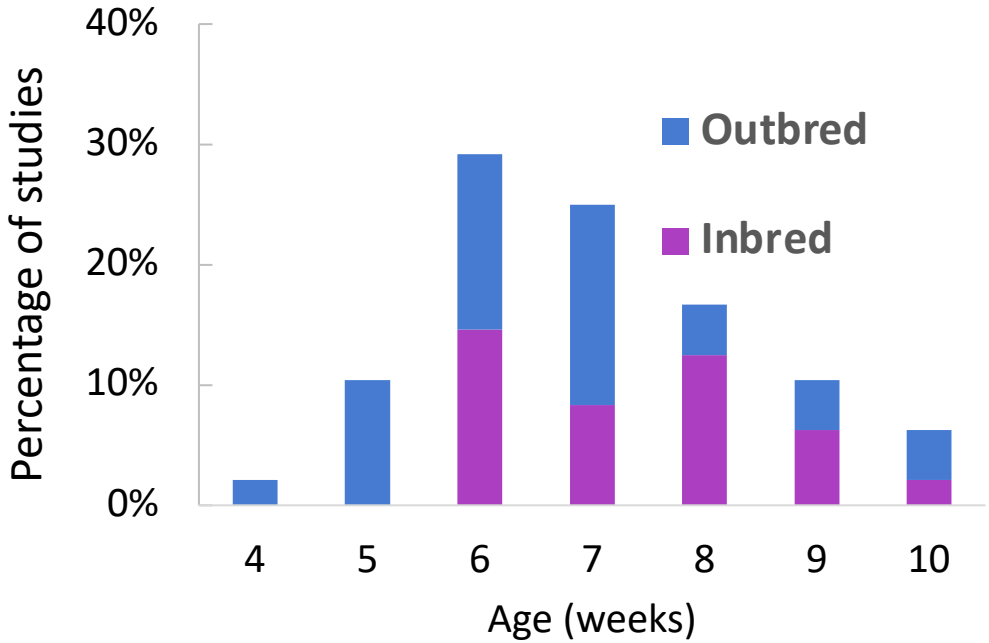


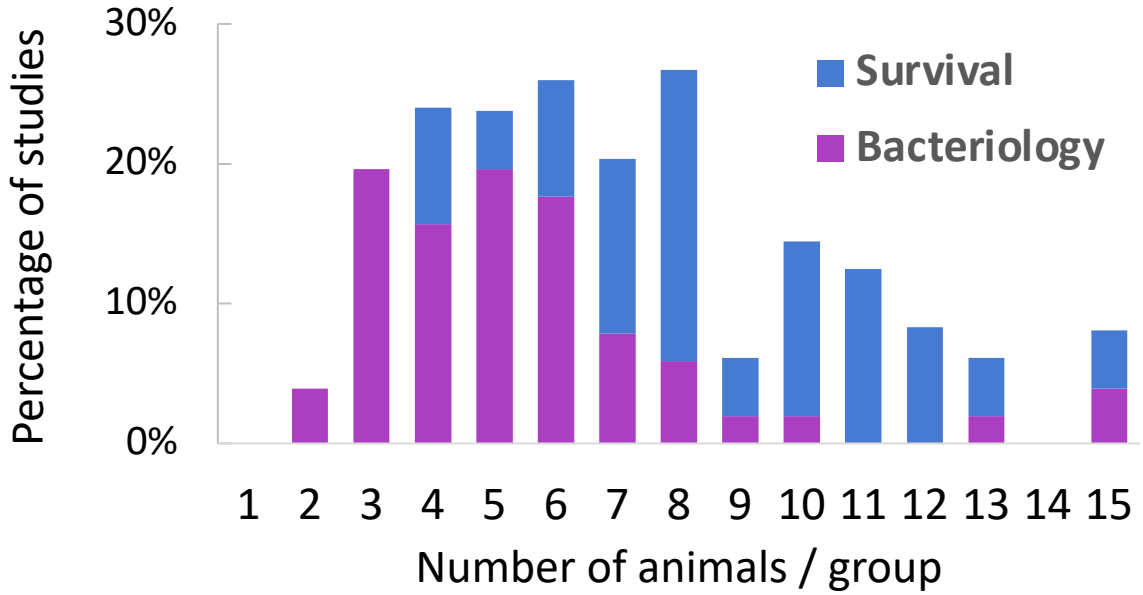
Institutional Protocol Review:



Identification of key variables: Mice



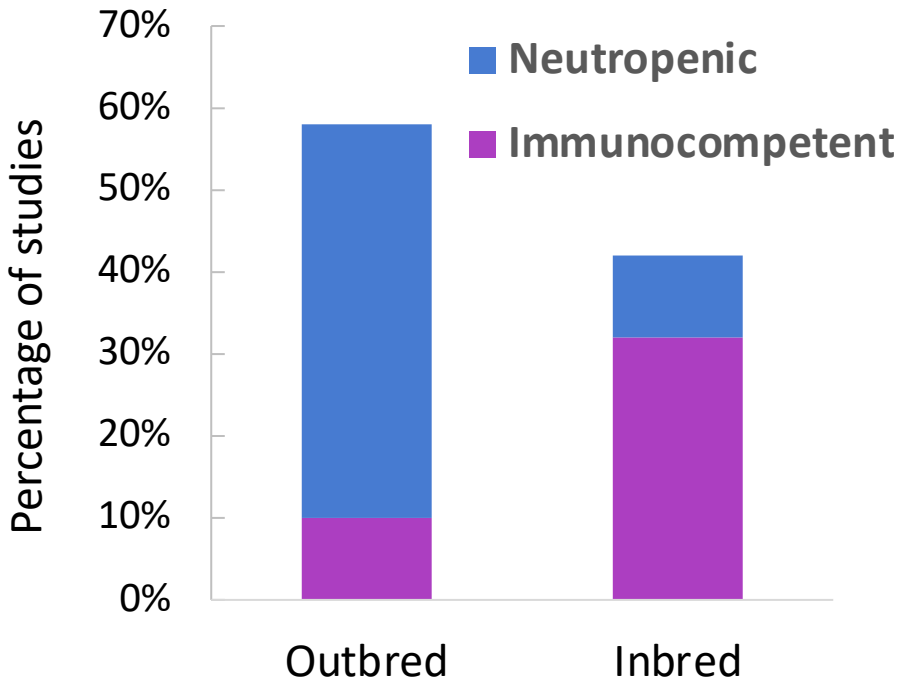


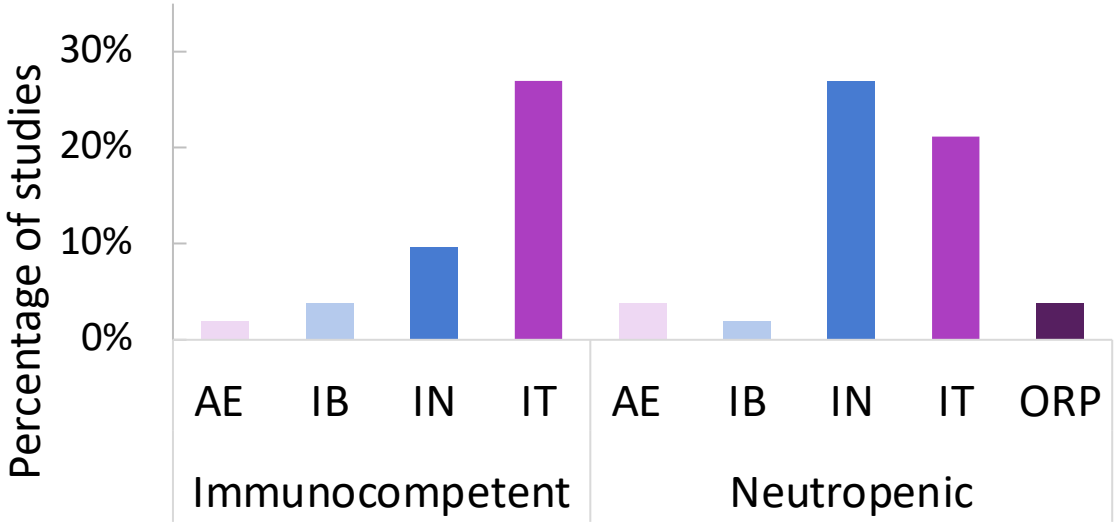


Proposed Standard

Female CD1 mice
6 ± 1 week old
5-6 mice per treatment group

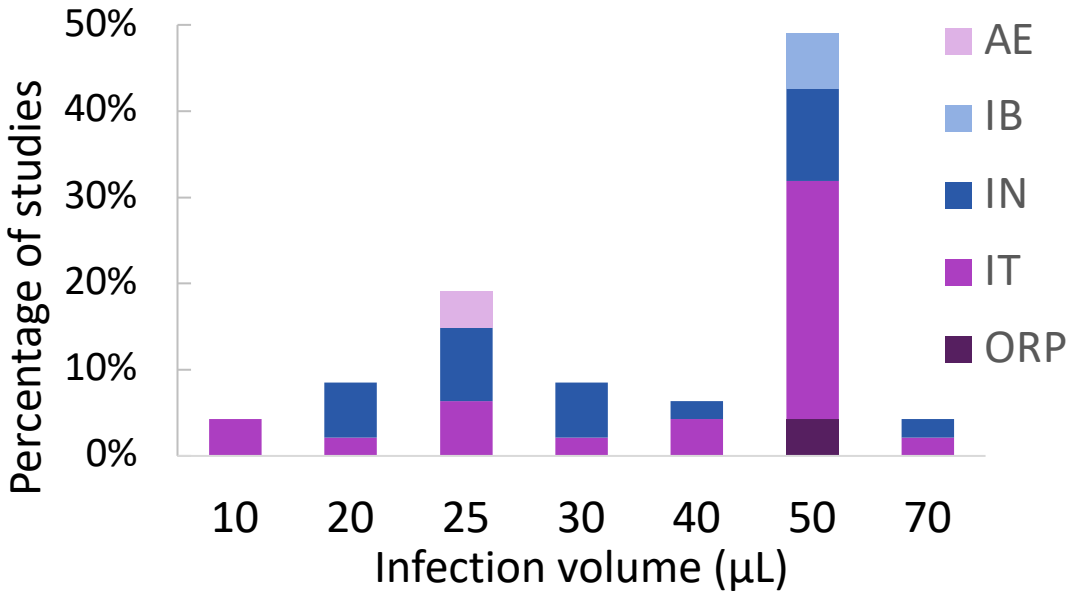
Identification of key variables: Inoculation



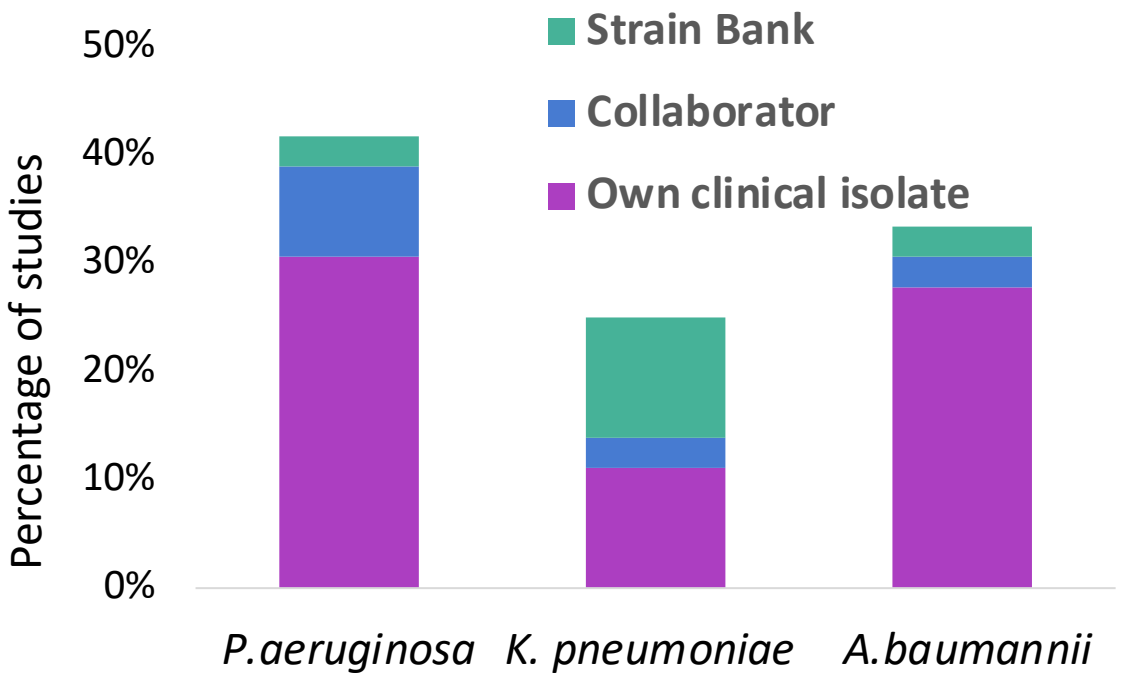


Proposed Standard

Immunosuppression
Intranasal inoculation
50 µl



Identification of key variables: Inoculum

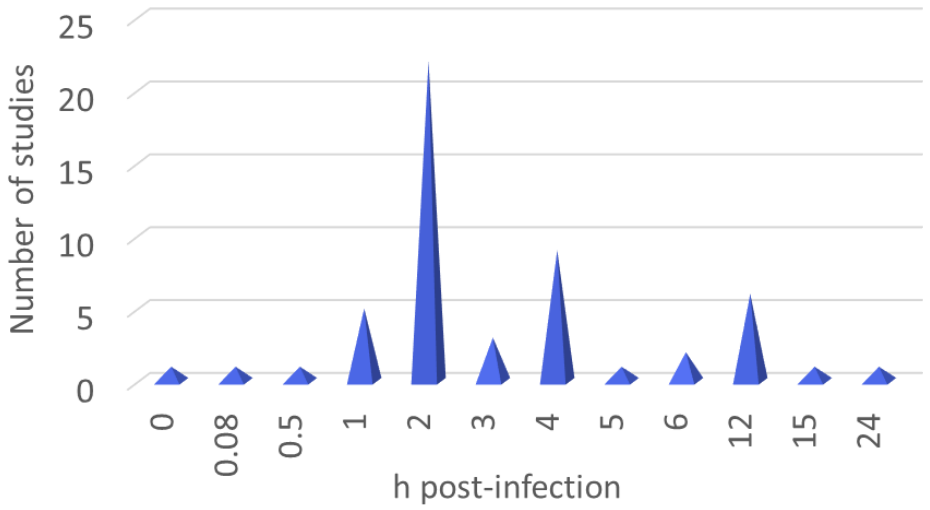


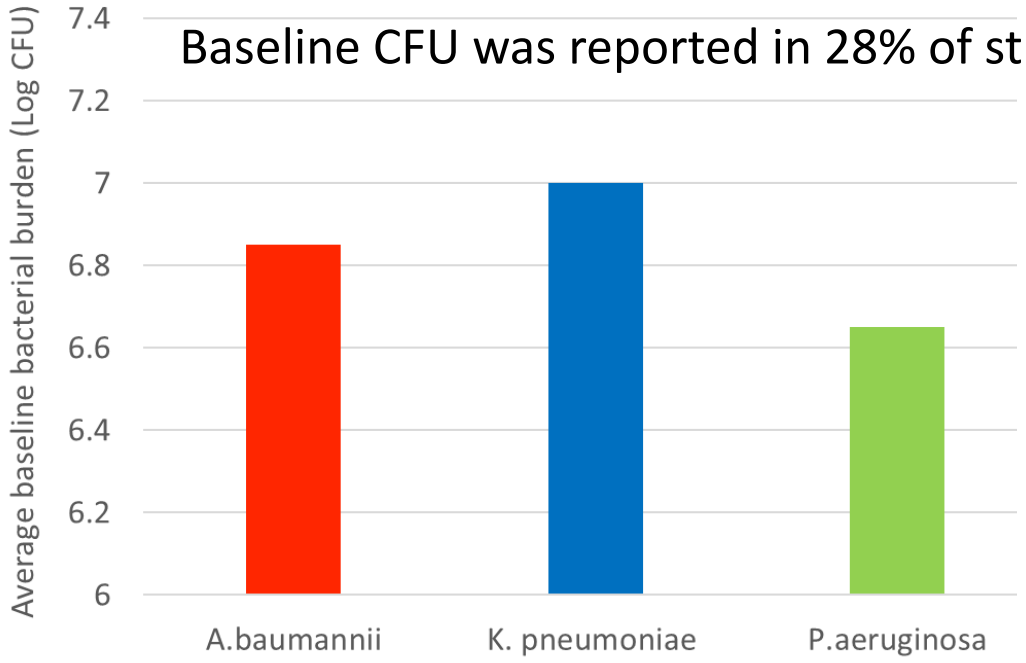
Culture stage	Number of studies
Subcultured to log. phase	13
Subcultured to early log. phase	4
Frozen log. phase stock	2
Subcultured to mid-log. phase	1

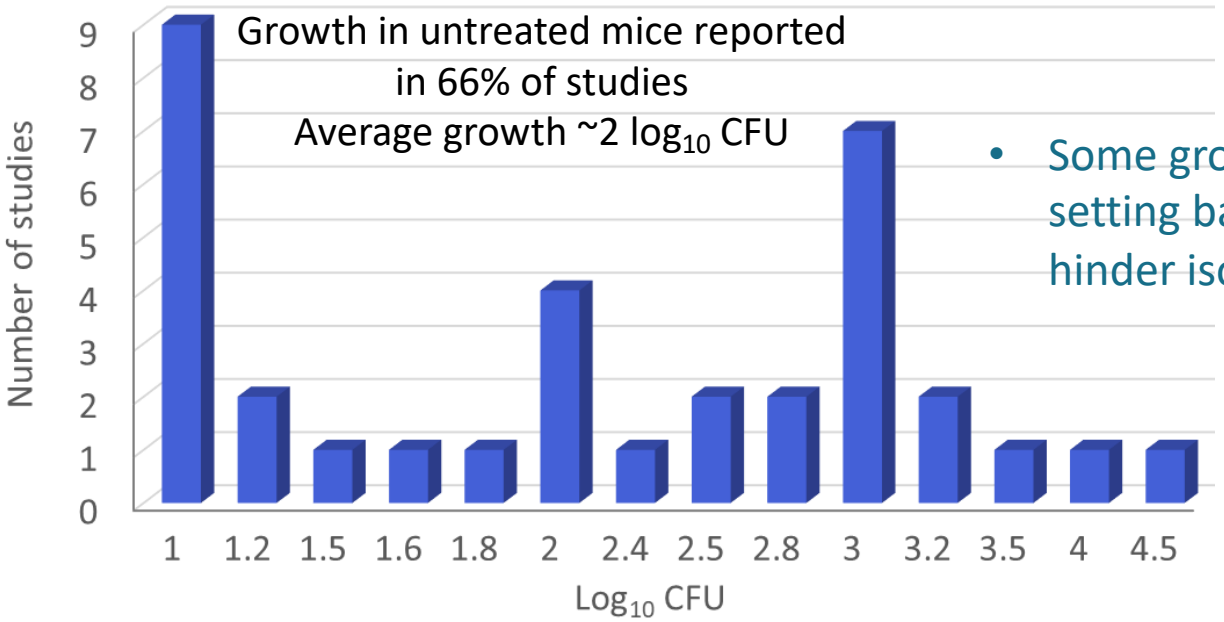
Proposed Standard

Exponential growth phase
Media and source as appropriate

Identification of key variables: Treatment start







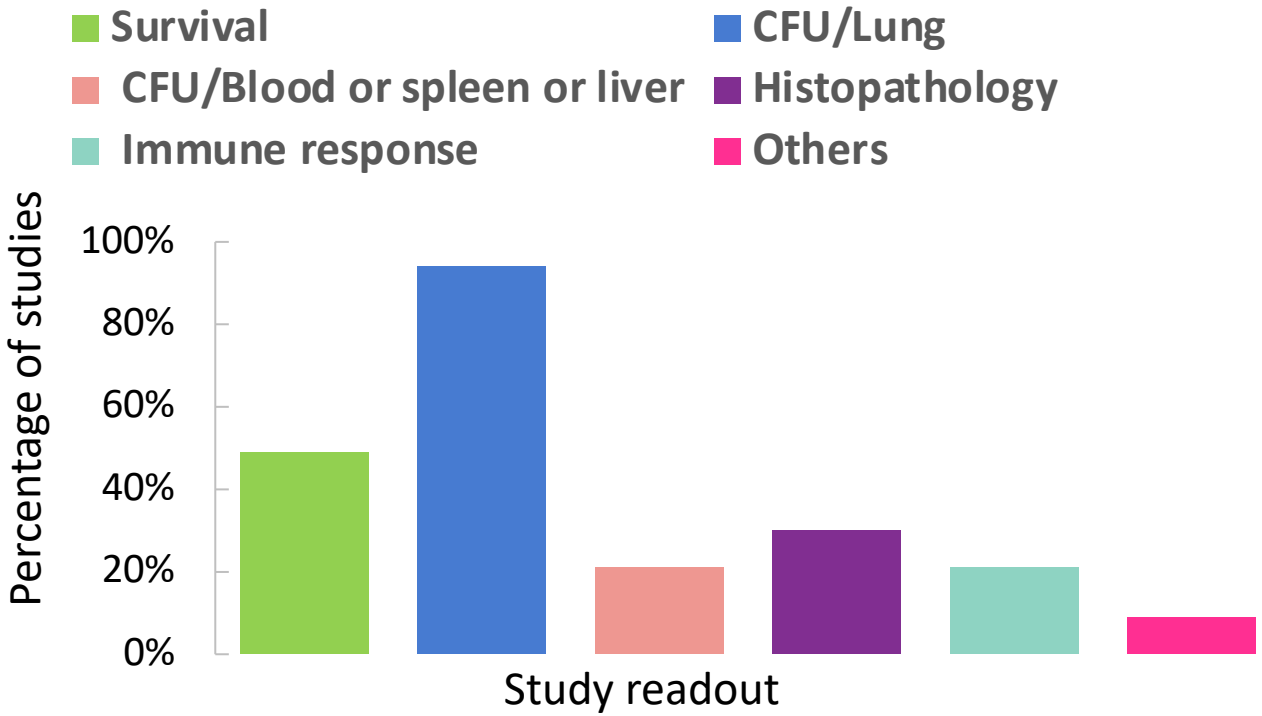
- Some growth is good but setting bar too high may hinder isolate selection

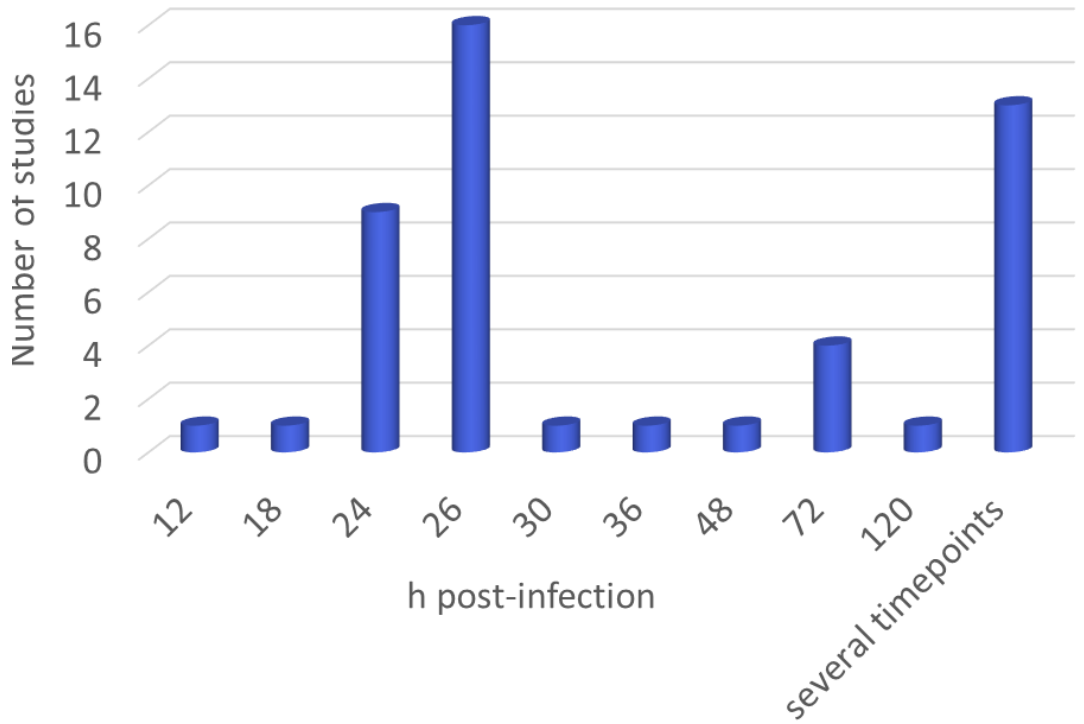
Proposed Standard

Start treatment 2h p.i.
6.0 – 6.5 (± 0.2) $\log_{10}$ CFU
≥1 $\log_{10}$ CFU growth

IMI AMR Accelerator Tackling antibiotic resistance together

Identification of key variables: Endpoints





Proposed Standard

CFU from lungs
End study at 26h p.i.

EXPERT WORKSHOP: Develop standardized murine model to evaluate treatments for AMR lung infections

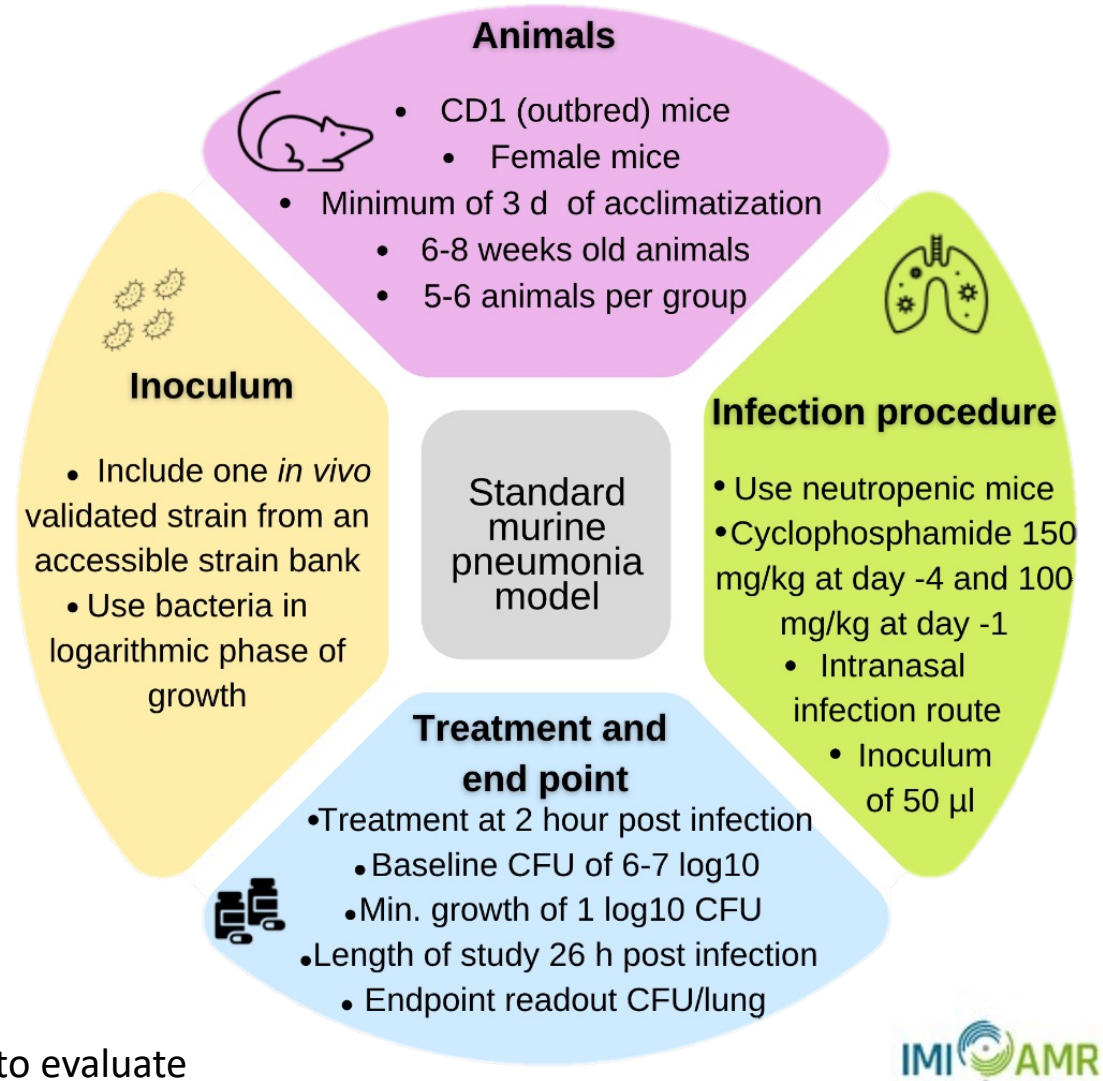
Day 1 (Tuesday, April 27th 2021): 15:00-19:00 CEST

Developing a standardized murine pneumonia model to characterize PK/PD of antibiotics

Day 2 (Wednesday, April 28th 2021): 15:00-19:00 CEST

Standard protocols for murine pneumonia models - beyond PK/PD

Expert Panel & Participant Survey
Selection of standardized variables



Rakel Arrazuria et.al. Expert Workshop Summary: Advancing towards a standardized murine model to evaluate treatments for AMR lung infections

Additional considerations

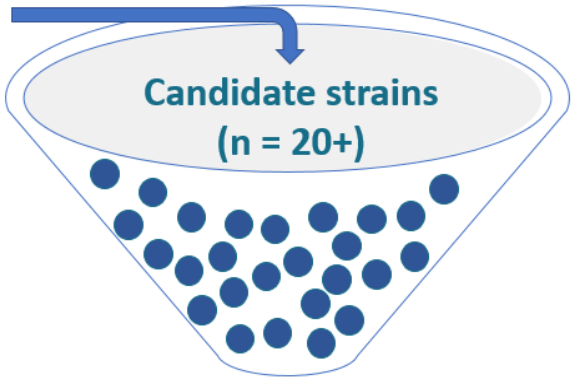
Animals 	• Use animals of the same sex consistently in the same study. After preliminary study test the effect in the other gender. • Use animals from the same vendor. • Adjust the number of animals to the power analysis if necessary. • Animal randomization is encouraged.
Inoculum 	• Time between inoculum preparation and its use in vivo should be short. • Ensure inoculum viability and growth consistency in the whole experiment.
Infection procedure 	• Anesthesia should be deep enough to allow the inoculum to settle in the lungs. • IT route should be considered for less pathogenic strains. • Inoculum > 20 µl should be used if lower inoculum volume is required.
Treatment and end point 	• If longer experimental endpoint (26 h) are needed for additional outputs (3-4 d), take several time points including 26 h. • Blinding the CFU counts if possible.

Validate standard pneumonia model

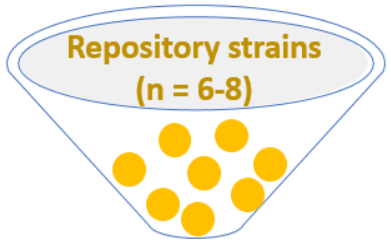
Bacterial Strains Selection Strategy

Partners, CCUG, GNA Now, DSMZ

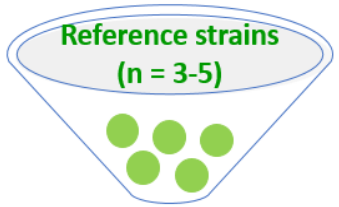
Chosen to test based on:
- Shareability
- Antibiogram
- Contemporary (if possible)
- Characterization (if available)

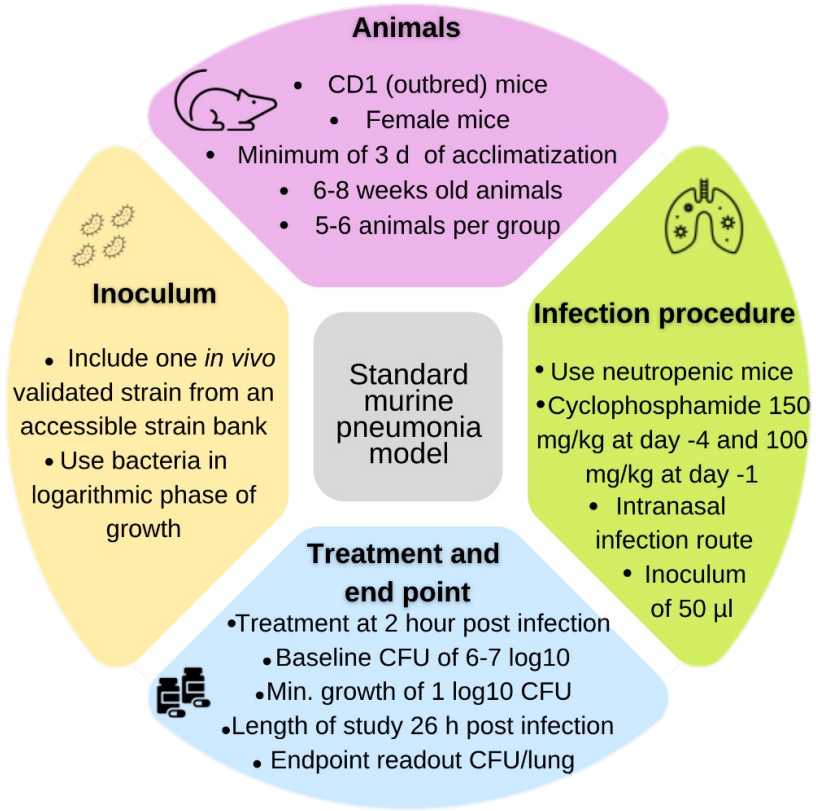


Candidate strains filtered by:
- Virulence
- Standard protocol criteria
- Mortality
- Expected +/- efficacy response



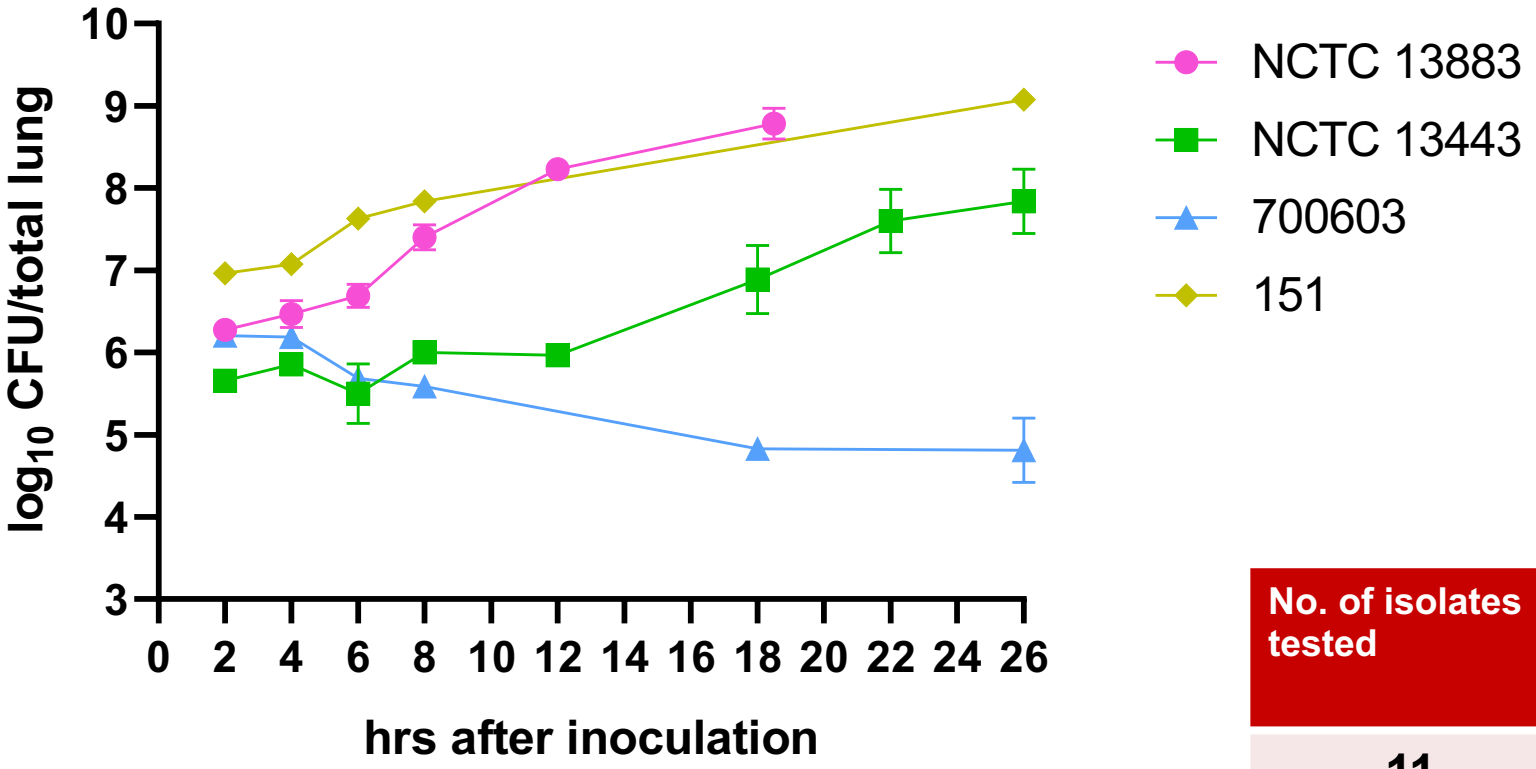
Ref strains chosen for:
- Inter-lab reproducibility
- PK/PD characterization





Validate standard pneumonia model

K. pneumoniae



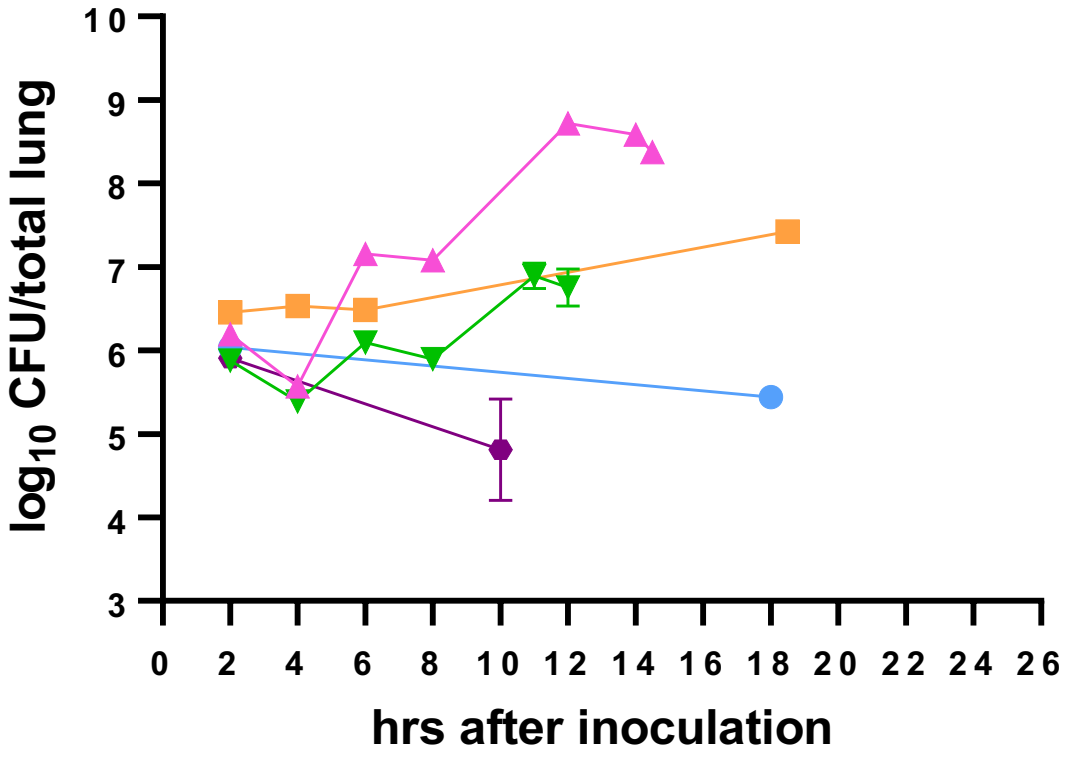
No. of isolates tested	Increase in bact. load < 1 log	Lethal <12hrs	Suitable strains identified
11	2	0	9

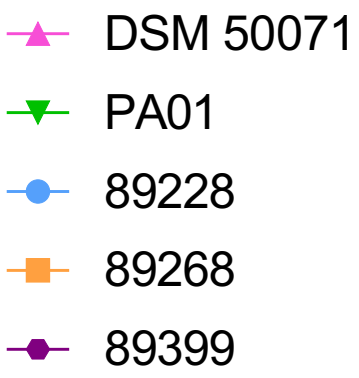
Poster: Jon Hansen et al

A standard protocol for the murine pneumonia model to evaluate treatments for AMR lung infections

Validate standard pneumonia model

P. aeruginosa





No. of isolates tested	Increase in bact. load < 1 log	Lethal <12hrs	Suitable strains identified
12	2	8	2

Poster: Jon Hansen et al

A standard protocol for the murine pneumonia model to evaluate treatments for AMR lung infections

Would you like to collaborate with COMBINE?

Contact us:
IMI-COMBINE@pei.de

Share your preclinical pneumonia data

Conduct validation studies in your lab
Share isolates for repository

Acknowledgement

COMBINE WP5 Members:

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SSI *in vivo* pharmacology team

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Sandra Bondo Jensen

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