

1 **Development of acute *Pseudomonas aeruginosa* and *Acinetobacter***
2 ***baumannii* lung mono-challenge models in mice using oropharyngeal**
3 **aspiration.**

4 Irene Jurado-Martín*,^{1,2} Chaoying Ma*,^{1,2} Nouran Rezk^{1,2}, Maite Sainz-Mejías,^{1,2}
5 Yueran Hou,^{1,2} John A. Baugh^{2,3} and Siobhán McClean¹

6 ¹ School of Biomedical and Biomolecular Science, University College Dublin, Dublin,
7 Ireland

8 ² UCD Conway Institute of Biomolecular & Biomedical Research, University College
9 Dublin, Dublin, Ireland

10 ³ UCD School of Medicine, University College Dublin, Belfield, Dublin, Ireland

11 * Both these authors contributed equally to this work.

12

13 Corresponding author: siobhan.mcclean@ucd.ie

14

15

16

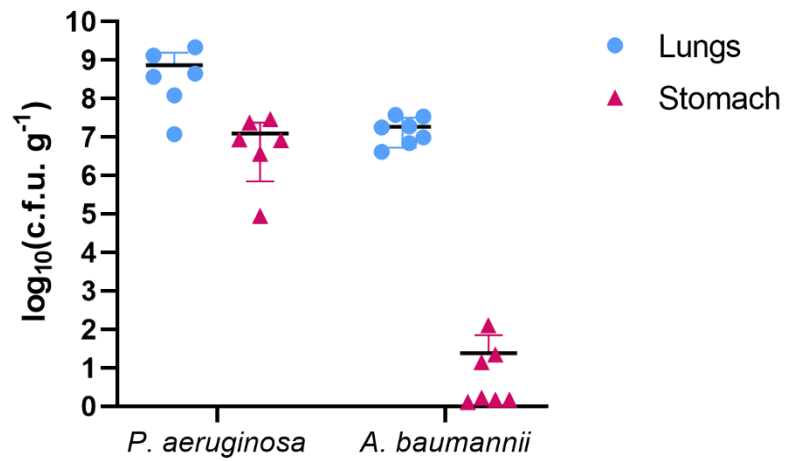
17

SUPPLEMENTARY MATERIAL

18

20 **Table S1.** Scoresheet used to evaluate mice severity after each procedure.

COMBINED POST PROCEDURAL / TRIAL SCORE SHEET (General & MGS combined)		07.10.20	
PI / Researcher	Siobhán McClean/Irene Jurado	Animal ID:-	Treatment Group:-
HPRA Project	AE18982/p209		
SCORE SHEET			
Appearance		Date/Time	Initials
0 Normal			
1 Slight piloerection / slight change in posture or respiration			
2 Moderate piloerection / moderate change in posture or resp			
3 Pronounced piloerection / pronounced change in posture or resp			
	Initial weight (g)		
Weight change:			
0 Normal			
1 Body wt <10% decrease			
2 Body wt 10-20% decrease			
3 Body wt >20% decrease			
Behaviour			
0 Normal			
1 Minor depression			
2 Decreased mobility or alertness / isolated			
3 Noticeably still / Precognatose / Restless / Vocalisation			
Hydration			
0 Normal			
1 5% dehydration - skin tents briefly, but returns to normal			
2 10% dehydration - Skin tents, and takes more than a few seconds to return			
3 15+% dehydration - skin tents and stays tented			
	Total (0-12)		
MOUSE GRIMACE SCALE (MGS)			
Orbital Tightening (0, 1, 2)			
Nose Bulge (0, 1, 2)			
Cheek Bulge (0, 1, 2)			
Ear Position (0, 1, 2)			
	Total		
BINARY OBSERVATIONS - Comments below (with date) if answer is Y or Not OK			
Analgesia administered subsequent to scoring (Y/N)			
Skin Lesions	Notify BMF staff if severe		
SCORE SHEET ACTIONS (FOR MODERATE BANDING)			
Score 0-1	Normal - No action required	Score	ACTIONS
Score 2-4	Repeat scoring in 6 hours	0-2	None
Score 5-7	Suffering - Administer analgesia/observe regularly/notify BMF staff (and DV by email)	3-4	Administer analgesia and score again in four hours
Signs of pain	Analgesia per protocol/notify BMF staff. Euthanasia for severe/intractable pain	5-6	Administer analgesia, notify DV by email and score again in four hours -> euthanise at that point if still scoring the same or higher
Animal also to be euthanased (unless DV consults if):			
a) Score of 3 in any single category, or b) Total score of greater than 7 or c) Otherwise indicated			
COMMENTS / TECHNICAL OFFICER OBSERVATIONS: Written (with date/time) on the back of the sheet and a tick placed			
here to indicate this has been done. Immediate euthanasia unless approved by DV to continue on trial. Notify DV by email.			

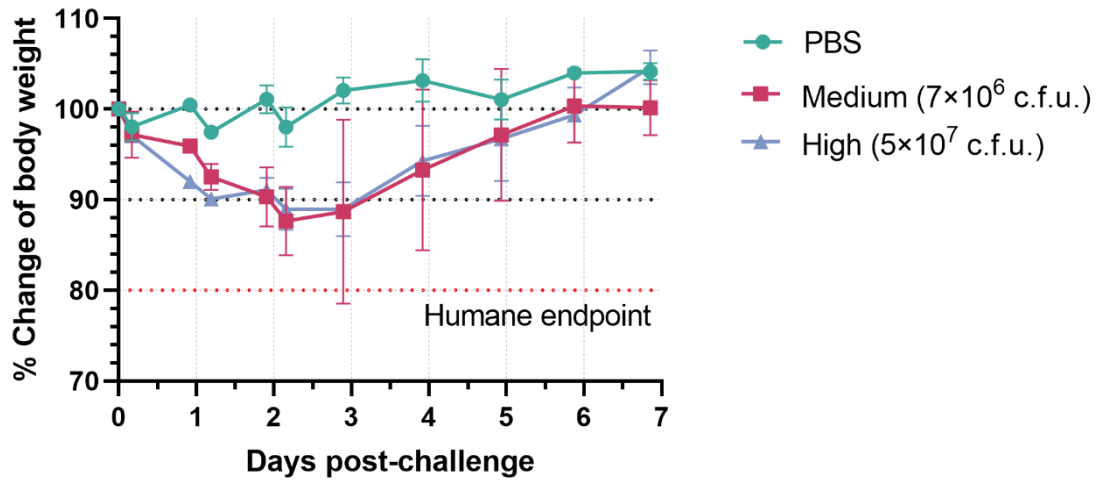


23

24 **Figure S1. Comparison of bioburden in the lungs and stomach of mice challenged with *P. aeruginosa***
 25 **or *A. baumannii*.** The graph compares the bioburdens obtained from an experiment performed by the same
 26 researcher, once optimisation studies were complete. *P. aeruginosa* bioburden is consistently higher than
 27 that of *A. baumannii*. Results are represented with the mean \pm SEM, with each point representing one
 28 mouse.

29

30



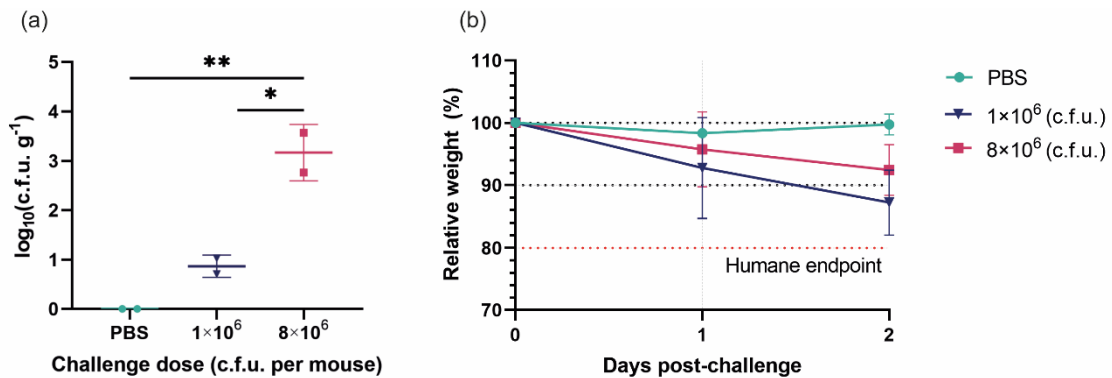
31

32 **Figure S2. Results of the acute *A. baumannii* pneumonia model 7 days after challenge.** Changes in
33 mice weight relative to that before the procedure. Each point represents the mean \pm SEM of the mice in
34 the group (n=2).

35

36

37



38

39 **Figure S3. Initial dose-finding pilot study performed acute *A. baumannii* pneumonia model 48 h**
40 **after challenge.** (a) Bioburden in the lungs of mice challenged with PBS, low, or medium bacterial
41 doses. Results are represented with the mean \pm SEM, with each point representing one mouse. (b)
42 Relative weight lost or gained by mice relative to that before the procedure. Each point represents the
43 mean \pm SEM of the mice in the group (n=2).

44