Tracking bacterial infection and treatment in *Galleria mellonella* using luminescent bacterium

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1 INTRODUCTION

Antimicrobial resistance is becoming a huge threat to the global population, with multi-drug resistance developing in multiple bacterial pathogens, highlighting the need for antimicrobial drug discovery.

Galleria mellonella (wax moth larvae) are widely used as an infection model system to study host-pathogen interactions and virulence, and are increasingly now used to determine the PK/PD, efficacy and toxicity of novel compounds. *G. mellonella* are known to be susceptible to 45 bacterial species, including ESKAPE pathogens, such as *Pseudomonas aeruginosa*.

G. mellonella possess an innate immune system comprising of a cellular and humoral response, similar to that seen in mammals. This similarity, coupled with the ease of maintenance, low cost, 37°C incubation, and ability to be used as a high throughput model system, makes *Galleria* an attractive *in vivo* system for efficacy and toxicity testing of novel compounds.

Biosystems Technology have developed research grade, genome sequenced, *G. mellonella* (TruLarv™), which are grown free of hormones and antibiotics. At KWS Biotest, a Charles River Company, we aimed to develop an infection model system using TruLarv™ and bioluminescent bacteria (Perkin Elmer) to demonstrate that antimicrobial efficacy and bacterial growth can be tracked using in-life, *in vivo* imaging system (IVIS, Perkin Elmer Lumina II) and GloMAX (Promega) technology.

2 MATERIALS AND METHODS

Our model system uses *G. mellonella* (TruLarv™) and luminescent *Pseudomonas aeruginosa* strain Xen41 (a derivative of PA01), which has been genetically engineered to express the *Photorhabdus luminescens lux* operon, to assess the efficacy of antimicrobial compounds.

Infections were performed using a Hamilton syringe with a 30G needle, with 10 µL injections of a lethal dose of luminescent bacterium (e.g. Xen41), and antimicrobial treatments, administered 2 hours post infection into an alternate proleg (Fig. 1).

DPBS infection and treatment controls (vehicle) were included in all studies.

Survival, melanisation, CFU burden (CFU/mL) and luminescence were monitored throughout the course of the infection.

In life bacterial luminescence was primarily measured using GloMAX technology, after an initial test using an *In Vivo I*maging System (IVIS) imaging.

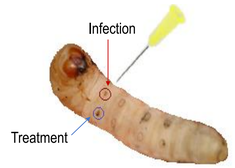

Fig. 1 – *Galleria mellonella* infection and antimicrobial treatment sites. Representation of the infection and treatment sites used during our study.

3 RESULTS

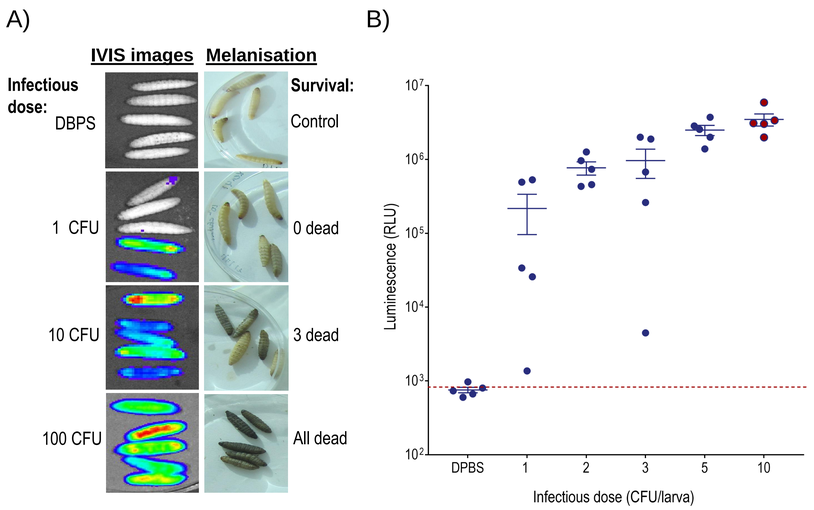

Fig. 2 - *P. aeruginosa* Xen41 infection can be tracked using IVIS and GloMAX technology. A) IVIS images after infection with 1, 10 or 100 CFU Xen41 after 16 hpi. IVIS images (left) at 16 hpi show degree of melanisation/survival (right) at each infectious dose. Higher degree of melanisation corresponds with a reduced survival and increased luminescence, seen with both IVIS and GloMAX technology. B) Scatter plot showing luminescence at 16 hpi (RLU), measured using GloMAX technology, of individual infected larvae; blue symbols – alive; red-filled symbols - dead. Dotted red line indicates base luminescence (RLU) seen with DPBS control.

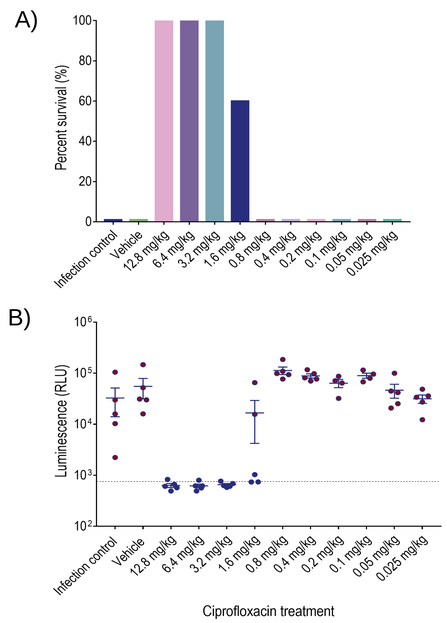

Fig. 3 – Survival and luminescence of *G. mellonella* following infection with 10^4 CFU *P. aeruginosa* Xen41 and treatment with ciprofloxacin. A) Percentage survival, 24 hpi, of infected *Galleria* following treatment 2 hpi with a range of ciprofloxacin concentrations. B) Luminescence emitted from infected and treated larva 24 hpi. Red-filled symbols – dead larvae, blue-filled symbols – alive; n = 5, ± SEM.

4 CONCLUSIONS

We have shown that luminescence signal correlates directly with melanisation, CFU/mL and survival rate of individual infected larvae, allowing us to gain a better understanding of the efficacy of antimicrobial compounds over time. The levels of luminescence exhibited by the Xen41 infected *Galleria* (between 10^5 - 10^6 RLU) showed a significant difference in luminescence signal when compared to treated (e.g. ciprofloxacin or meropenem) or vehicle control groups which display a luminescence signal between 10^2 - 10^3 RLU.

Our *Galleria* time-kill curve analysis have shown comparable luminescence and CFU/mL results, both showing a dose response with increasing antibiotic concentrations, with the decline in luminescence seen being directly comparable with the drop in bacterial burden within individual larvae

Our system can be applied to other bacterial pathogens and bacterial luminescent strains to assess the efficacy of novel antimicrobial compounds, prior to their use mammalian *in vivo* model systems. We envisage the use of *G. mellonella* TruLarv™ and luminescent bacterium will provide a cost effective pre-*in vivo* model system for toxicity and efficacy testing of novel compounds, and will subsequently reduce the number of animals used in subsequent studies and time taken for initial *in vitro* screening.

If you are developing drugs aimed at treating bacterial pathogens that are a growing public health concern, give us a call or get in touch with our Global Discovery Infection services.

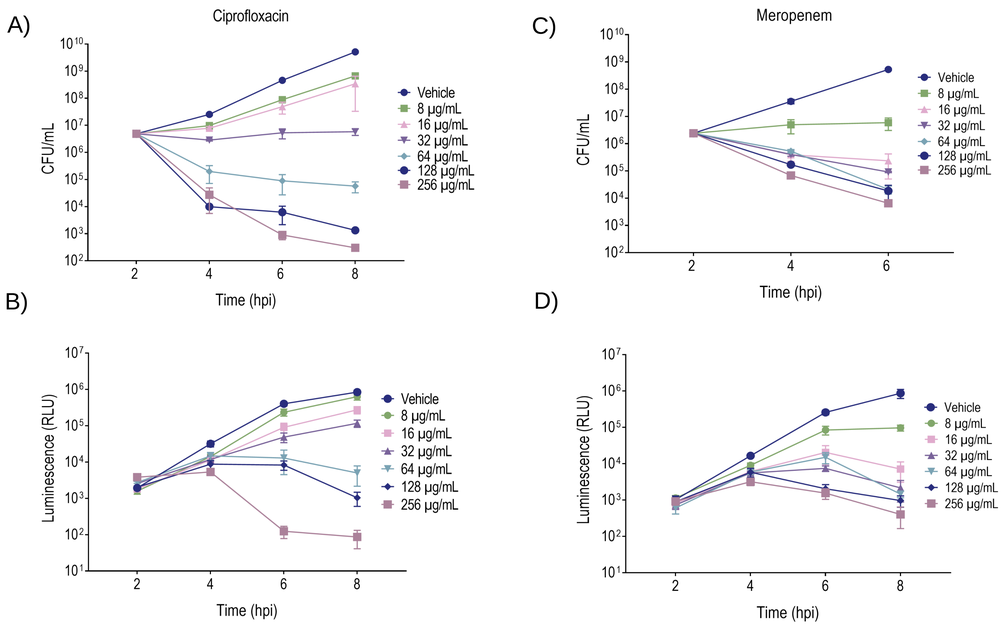

Fig. 4 – Antibiotic time-kill curves can be conducted within *G. mellonella* and monitored using GloMAX technology. *G. mellonella* were infected with 10 µL 10^8 CFU/mL Xen41 and treated 2 hpi with a range of ciprofloxacin (A and B) or meropenem (C and D) concentrations and monitored till 8 hpi. A and C) Bacterial burden (CFU/mL) following 2 hpi treatment with ciprofloxacin (A) or meropenem (C). B and D) Change in luminescence (RLU) following ciprofloxacin (B) or meropenem (D) treatment. Luminescence at 2 hpi was measured prior to antibiotic treatment. Results show mean luminescence ± SEM (n = 3 for CFU/mL and n = 5-8 for luminescence readings) for each treatment group after normalisation against base luminescence seen with DPBS infection control.