ImQuest BioSciences

Micro*SENS* Technical Note

Optimization of *Galleria mellonella* Larvae Model to Assess the Effect of Antimicrobial Agents on ESKAPE Pathogen Infection

Introduction

Murine models are commonly used to assess the activity of antimicrobial agents to treat infection. While they are a relatively inexpensive option compared to large animal models, the number of animals required to obtain statistically significant data results in significant increases in assay cost. Additionally, with an increased number of companies developing host directed and immune modulating products, which are not well suited to traditional *in vitro* screening techniques, screening endeavors in the murine model could be prohibitively expensive. To mitigate these issues the greater wax moth *Galleria mellonella* (*G. mellonella*) has become increasingly popular for several reasons: (1) The caterpillar larvae of *G. mellonella* are inexpensive and easy to maintain, (2) they do not require any special laboratory equipment, (3) they do not require IACUC approval and (4) their short half-life makes them ideal for compound screening. Importantly, their innate immune response has similarities to the human innate immune response so this model can be utilized effectively to assess the impact of innate immune modulators. Thus, to provide a valuable and cost-effective model to screen novel, antibacterial compounds, ImQuest's microbiology team has developed and optimized the *G. mellonella* model and now offers this model as an important component of our microbiology services.

Methods and Results

Our model development and optimization included the evaluation of representative wild type and resistant ESKAPE bacteria. Prior to infection, larvae were starved for 24 hours by placing them in petri dishes overnight at 30°C. For each strain of bacteria, five groups (4 bacterial inoculum groups and a DPBS negative control group) were evaluated. Each group was comprised of 10 larvae and larvae sizes per group were standardized. On the day of infection, each larva was inoculated with 10 µL of either the designated bacterial inoculum or DPBS into one of the larvae pro-legs. The larvae were incubated at 37°C for 5 days. Each day, each larva was observed for mortality, indicated by dark pigmentation (melanization), reduced activity (movement with and without stimulation) and survival.

Data were obtained with representative Gram-negative and Gram-positive strains of bacteria. *P. aeruginosa* ATCC strain 27853, a quality control strain, and *P. aeruginosa* ATCC strain 29260/PA-103, a virulence factor exotoxin A producing strain, were assessed as representative Gram-negative organisms. For each strain inoculums containing 10^7 CFU to 10^4 CFU were evaluated. By day 2 all larvae were dead, for both strains of *P. aeruginosa*. Data are presented in Figures 1A and Figure 1B.

Figure 1A

Survival of G. mellonella infected with P. aeruginosa strain 27853 (inoculums of 10^4 to 10^7 CFU)

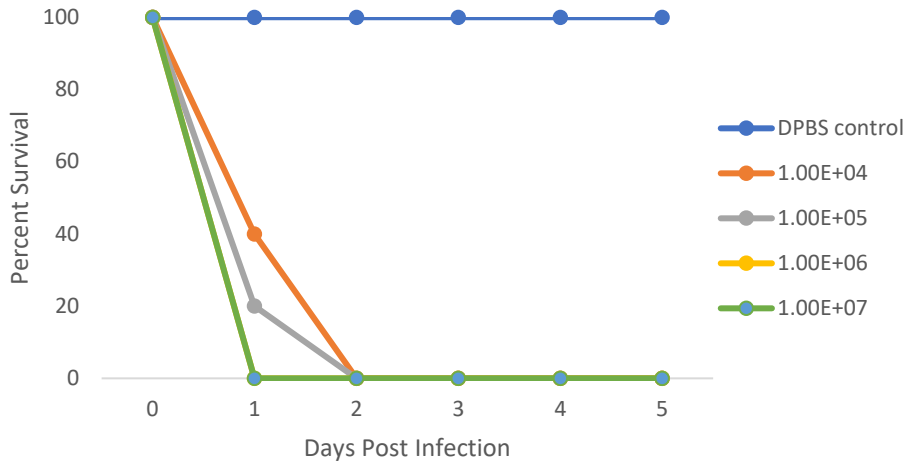
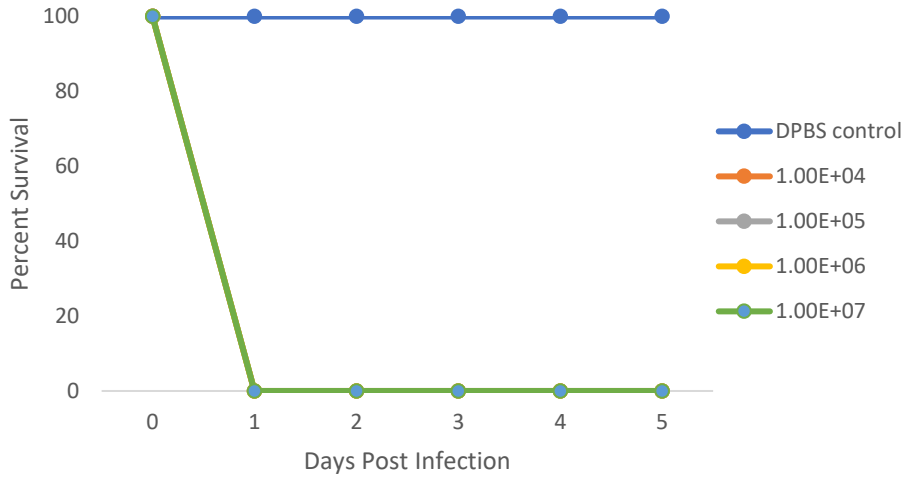


Figure 1B

Survival of G. mellonella infected with P. aeruginosa strain 29260 (inoculums of 10^4 to 10^7 CFU)



The experiment was repeated using inoculums of 10^4 to 10^1 CFU and 50% lethality of *P. aeruginosa* strain 27853 was determined to be at < 24 hours, < 24 hours, 5 days and >5 days for inoculums of 10^4 CFU, 10^3 CFU, 10^2 CFU and 10^1 CFU, respectively. Fifty percent (50%) lethality for each inoculum of *P. aeruginosa* strain 29260 was less than 24 hours. Data are presented in Figure 1C and Figure 1D.

Figure 1C

Survival of G. mellonella infected with P. aeruginosa strain 27853 (inoculums of 10^7 to 10^4 CFU)

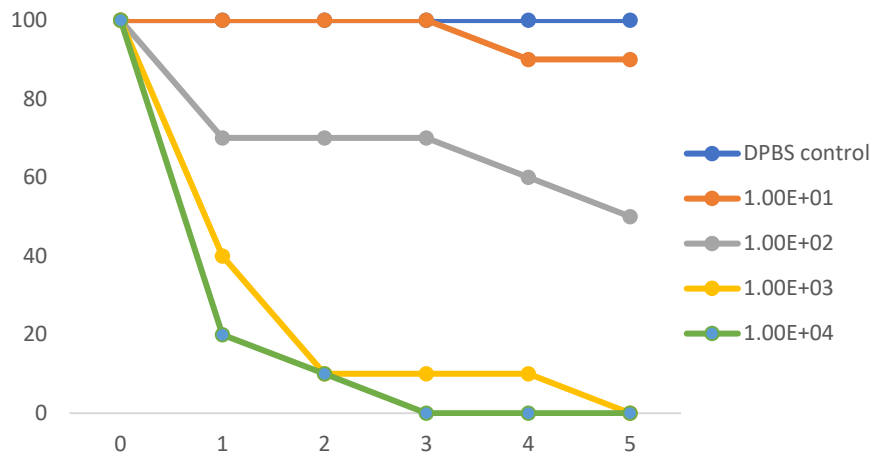
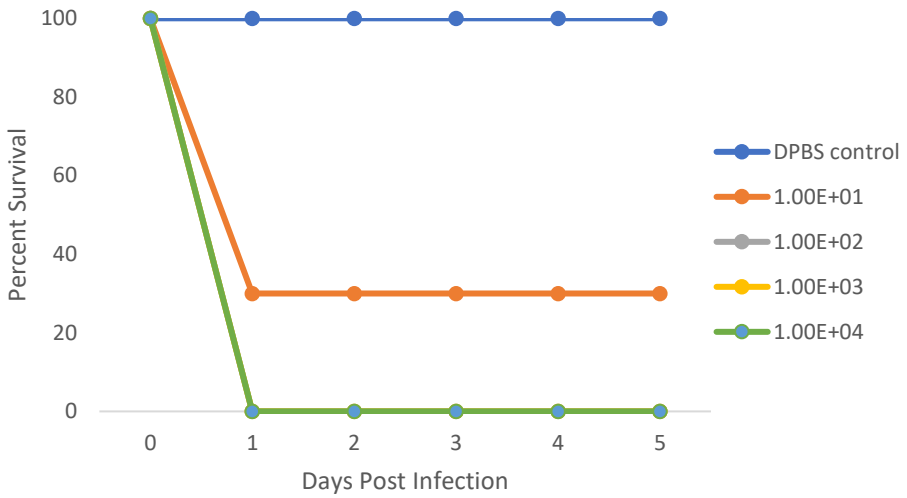


Figure 1D

Survival of G. mellonella infected with P. aeruginosa strain 29260 (inoculums of 10^7 to 10^4 CFU)



S. aureus ATCC strain 29213, a quality control strain, and *S. aureus* ATCC strain 43300, a methicillin and oxacillin-resistant clinical strain, were assessed as representative Gram-positive strains. For each strain, inoculums containing 10^7 CFU to 10^4 CFU were evaluated. Fifty percent (50%) lethality for *S. aureus* strain 29213 was at <24 hours for the 10^7 inoculum and >5 days, for inoculums of 10^6 CFU, 10^5 CFU and 10^4 CFU.. Fifty percent (50%) lethality for *S. aureus* strain 43300 was >5 days for all inoculums except for 10^7 where it was determined to be between day 1 and day 2. Data are presented in Figure 2A and Figure 2B.

Figure 2A

Survival of G. mellonella infected with S. aureus strain 29213 (inoculums of 10^4 to 10^7 CFU)

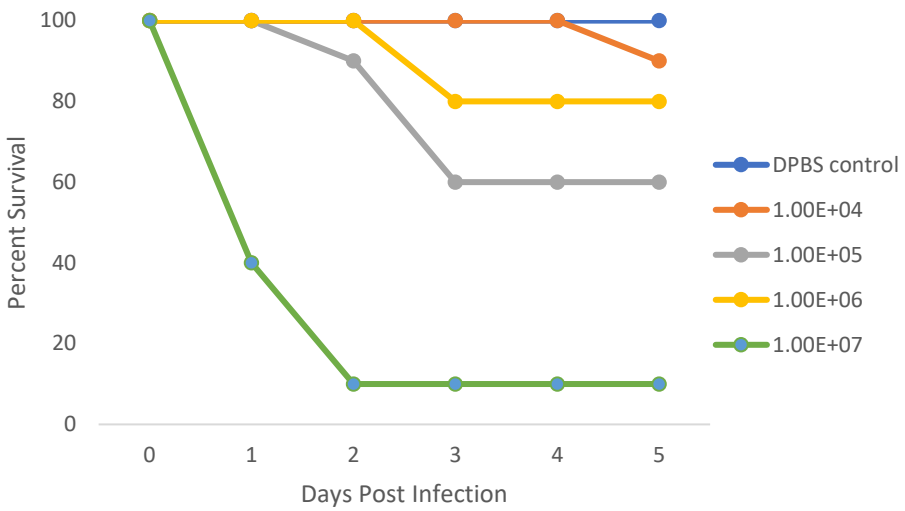
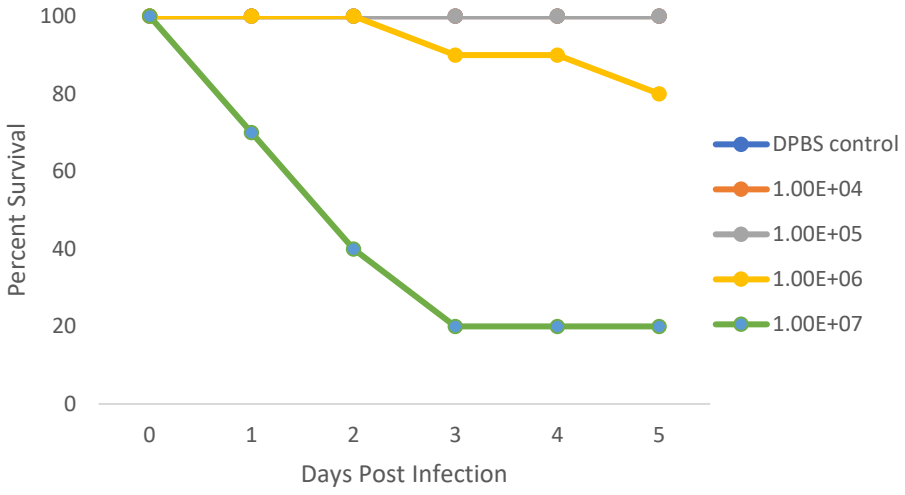


Figure 2B

Survival of G. mellonella infected with S. aureus strain 43300 (inoculums of 10^4 to 10^7 CFU)

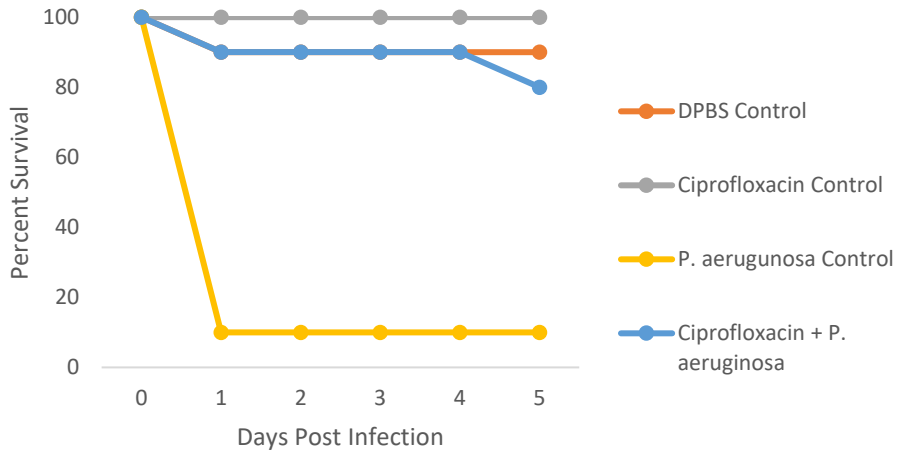


Utilizing the data generated from the survival curves, assays to assess the activity of control antibiotics against each organism were performed. Larvae were inoculated with a selected dose of bacteria that resulted in increasing mortality over a three-day assay timeframe. The antibiotics were dosed between 30 minutes and 1 hour following bacterial inoculation. A DPBS only, antibiotic only and bacterial inoculum only controls were assessed in parallel.

Larvae were inoculated with *P. aeruginosa* strain 27853 at 1 x 10^3 CFU and dosed with 5 μg of ciprofloxacin in a volume of 10 μL. At 24 hours post-infection, the *P. aeruginosa* infected group had a 10% survival rate. This rate of survival was maintained over 5 days. The DPBS control group, ciprofloxacin only control group and the ciprofloxacin + *P. aeruginosa* infection group had 90 to 100% survival through day 4 which dropped to 80% in the ciprofloxacin + *P. aeruginosa* infection group on day 5. Data are presented in Figure 3.

Figure 3

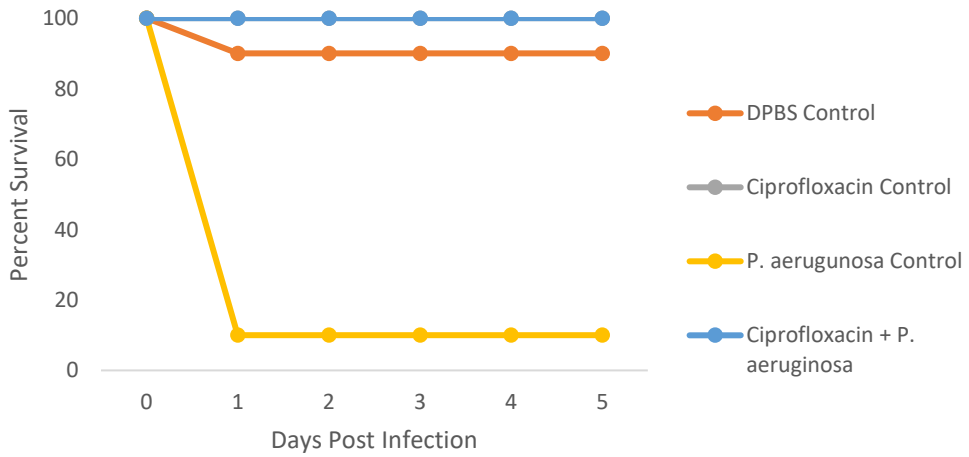
Evaluation of ciprofloxacin treatment in G. mellonella following infection with P. aeruginosa strain 27853



Larvae were also inoculated with *P. aeruginosa* strain 29260 at 1 x 10^1 CFU and dosed with 5 μg of ciprofloxacin in a volume of 10 μL. At 24 hours post-infection, the *P. aeruginosa* infected group had a 10% survival rate, which continued through day 5. The DPBS control group and ciprofloxacin only control group had 90% survival through day 5 and the ciprofloxacin + *P. aeruginosa* infection group maintained 100% survival through day 5. Data are presented in Figure 4.

Figure 4

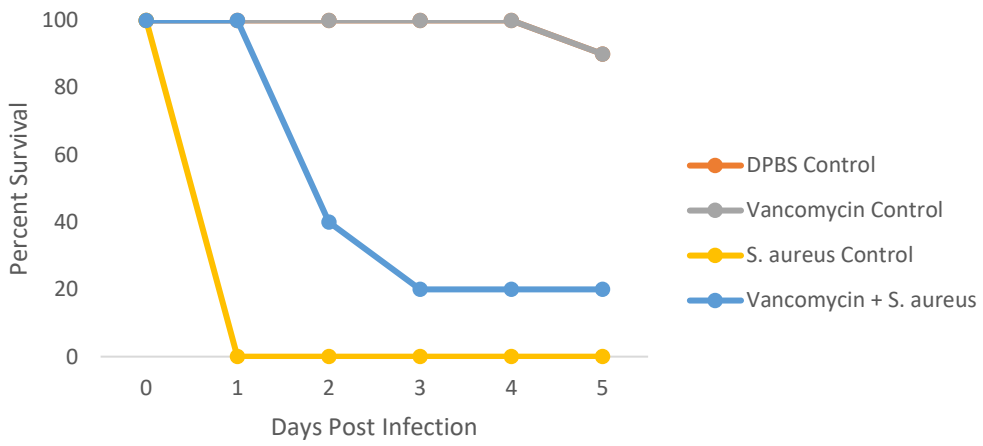
Evaluation of ciprofloxacin treatment in G. mellonella following infection with P. aeruginosa strain 29260



Larvae were inoculated with *S. aureus* strain 29213 at 1 x 10^7 CFU and dosed with 5 μg of vancomycin in a volume of 10 μL. At 24 hours post-infection, the *S. aureus* infected group had 0% survival. The DPBS control group and vancomycin only control group had 100% survival through day 4 which dropped to 90% on day 5. The vancomycin + *S. aureus* infected group had 100% survival at 24 hours, but this dropped to 40% survival by day 2 and only 20% survival by day 3. Data are presented in Figure 5.

Figure 5

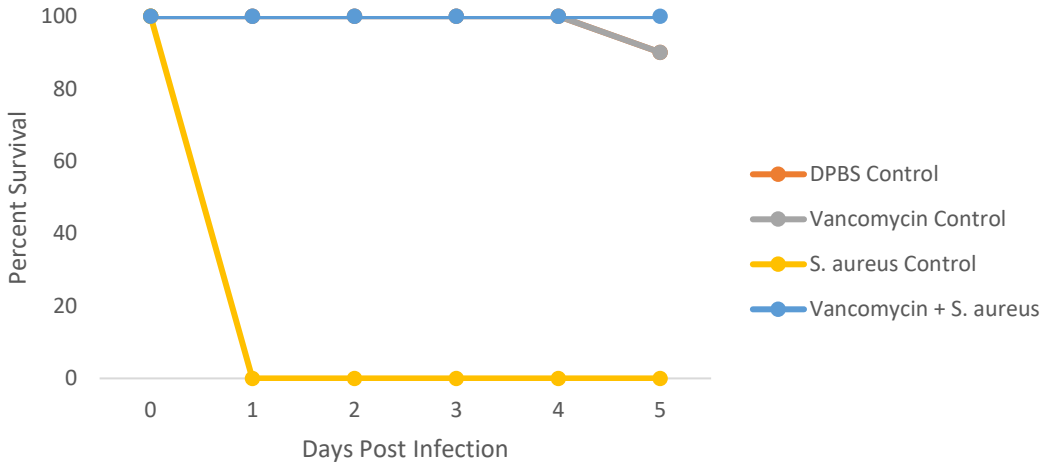
Evaluation of vancomycin treatment in G. mellonella following infection with S. aureus strain 29213



Larvae were inoculated with *S. aureus* strain 43300 at 10^7 CFU and dosed with 5 μg of vancomycin in a volume of 10 μL. At 24 hours post-infection, the bacterial infected group had a 0% survival rate. The DPBS control group and vancomycin only control group had 100% survival through day 4 which dropped to approximately 90% by day 5. The vancomycin + *S. aureus* infected group had 100% survival for 5 days following infection. Data are presented in Figure 6.

Figure 6

Evaluation of vancomycin treatment in G. mellonella following infection with S. aureus strain 43300



Discussion

ImQuest BioScience's microbiology team has developed and optimized the *G. mellonella in vivo* model to assess the efficacy of novel antimicrobial agents. A variety of representative wild type and resistant ESKAPE pathogens were tested and the *in vivo* model can be utilized to evaluate the effects of antibiotics on both Gram-positive and Gram-negative organisms. It can be utilized to assess the efficacy of novel antimicrobial agents identified in primary screening in a rapid and cost-effective manner prior to transitioning to more expensive murine or large animal models of infection. Additionally, due to the conserved innate immune response in these organisms, the *in vivo* assay is ideally suited to assess novel host directed and immune modulating agents. This assay serves to enhance ImQuest's Micro*SENS* platform which includes preclinical services necessary for the discovery and IND-directed development of novel anti-infective agents.

About ImQuest BioSciences

ImQuest BioSciences is a preclinical contract research and development company with decades of experience evaluating the potential of novel pharmaceutical products, with specific expertise in services for the development of antiviral, antimicrobial and anticancer products. We are dedicated to earning our client's trust through collaboration, unwavering commitment to quality science and consistent and effective communication.

References

Tsai, Catherine Jia-Yun, San Loh, Jacelyn Mei, Proft, Thomas. *Galleria mellonella* infection models for the study of bacterial diseases and for antimicrobial drug testing. Virulence. 2106 Apr; 7(3): 214-229

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