Apr 22nd, 9:45 AM

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USE OF *GALLERIA MELLONELLA* LARVAE FOR ASSESSING *PSEUDOMONAS AERUGINOSA* VIRULENCE

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Pseudomonas aeruginosa is a gram-negative opportunistic pathogen that is ubiquitous in the environment. Due to its multi-drug resistance mechanisms and virulence factors, *P. aeruginosa* has become a growing concern in the health care community and can be life-threatening in compromised individuals. High-throughput *in vivo* models are needed to assess the virulence of the wide variety of *P. aeruginosa* strains. *Galleria mellonella* has several advantages as a model organism in bacterial virulence studies, such as its affordability, minimal ethical restrictions, and ease of use. Our preliminary data indicated a low correlation (R^2: 0.33) between the lethal dose for 50% mortality in mice (mLD_{50}) and 50% mortality at 18 hours post-infection in *G. mellonella* (gLD_{50}) infected with *P. aeruginosa* bloodstream isolates. Also, poor correlation ($R^2 = 0.34$) was noted between the preliminary data's two biological replicates, indicating a problem with reproducibility. In this study, the protocol for virulence assays using *G. mellonella* as a model for *P. aeruginosa* infections was optimized and the sources of variability and unintended deaths in negative controls of preliminary studies were investigated. Injection site and depth, microbial contamination, and the impact of *G. mellonella* weight were investigated to determine if these factors created variation in preliminary experimentation. Mortality checks were changed to a 24-hour time course starting at 8 hours post-infection and continuing every hour until 24 hours post-infection or until 100% mortality was achieved. Five bacterial doses were checked, and the lethal time to achieve 50% mortality in *G. mellonella* (gLT_{50}) was determined for each dose. Exponential decay curves were fit to colony-forming units (CFUs) vs. gLT_{50}s plots, allowing for an accurate strain to strain comparisons at ~2,000 CFUs. The new methodology was then used to assess the relative gLT_{50} of ~49 strains of *P. aeruginosa.* The virulence assay retest demonstrated an increase in correlation between gLT_{50} and mLD_{50} (R^2: 0.61). These findings suggest that *G. mellonella* is a promising model organism for studying bacterial infections when utilizing our time course and dose-response curve protocol.