An Invertebrate Model to Study *Clostridioides difficile*

UNIVERSITY of HOUSTON COLLEGE OF PHARMACY

Poster # 1

Alnezary FS, Almutairi MS, Fallatah SB, Alam MJ, Begum K, Lancaster C, Gonzales-Luna AJ, Garey KW

Department of Pharmacy Practice and Translational Research, University of Houston College of Pharmacy

Contact Information:
Faris Alnezary
University of Houston
College of Pharmacy
Phone: (713) 743-1239
Email: falnezar@central.uh.edu

BACKGROUND

- *Clostridioides difficile* infection (CDI) is an urgent public health threat worldwide and a significant financial healthcare burden (1)
- Primary CDI treatments include vancomycin or fidaxomicin; however, disease recurrence after antibiotic therapy is increasing, which makes development of novel therapeutics is essential for treatment of CDI (2)
- Several animal models have been developed to study various aspects of CDI, including *C. difficile* pathophysiology, colonization, recurrence, efficacy testing of new antibiotics and the impact of strain variability (3)
- Animals that have been utilized to study CDI include mice, hamsters, rats, rabbits, hares, guinea pigs, and prairie dogs (3)
- Conducting experiments with these infection models is costly, time-consuming, and require extensive ethical consideration
- The invertebrate model *Galleria mellonella* has become an attractive alternative to other *in vivo* models in infectious diseases-related research, including bacterial and fungal virulence, viral infections, and antimicrobial screening and testing (4)
- This popularity is attributed to its low cost, short life cycle, easy handing, and simple ethical considerations (4)
- The data supporting the use of *G. mellonella* to study CDI is limited

OBJECTIVE

- This study investigated the feasibility of using *G. mellonella* as a surrogate insect model to study CDI pathogenesis

METHODS

Development stage

- **Median lethal dose (LD50) :** Larvae were gavaged (force fed) with $1x10^{2-6}$ colony forming units (CFU) of *C. difficile* ribotype (RT) 014-020 strain (MT-5313)
- **Duration of pre-incubation:** Larvae were gavaged with $1x10^5$ CFU of *C. difficile* RT 014-020 and Phosphate-buffered saline (PBS). The experiment was done on fresh (0-7 days) and old (>7days) larvae
- **Optimal growth temperatures:** Larvae were gavaged with $1x10^5$ CFU of *C. difficile* RT 014-020 and incubated at 30°C and 37°C and monitored for 120 hours

Validation stage

- *G. mellonella* larvae (n=10/experiment) were gavaged with $1x10^5$ CFU using several *C. difficile* RT strains (RT027, RT106, RT014/020, RT012)
- Larvae were assigned into the following arms and experiments were repeated in duplicate:
  1. Negative control (PBS only), n = 20
  2. Positive control (clinical *C. difficile* isolates; n = 10 for each strain), n = 180
  3. Positive control (standard *C. difficile* isolates (R20291, CD630); n = 10 for each strain), n = 40
- In all experiments, larvae were kept at 37°C post-infection and monitored daily for 120 hours for survival

Figure 1. Gavaging of *G. mellonella*

RESULTS

Figure 2. Effect of duration of pre-incubation on larvae survival (n=80)

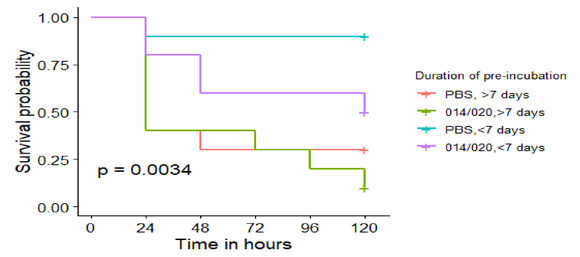

Figure 3. Effect of temperature on larvae survival (n=100)

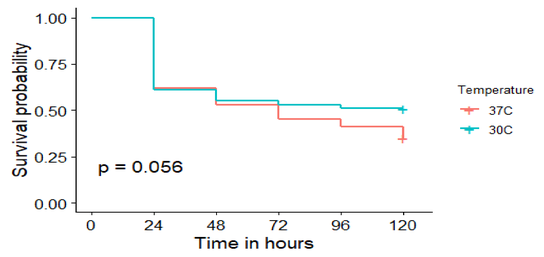

Figure 4. Effect of different *C. difficile* strains on larvae survival (n=40)

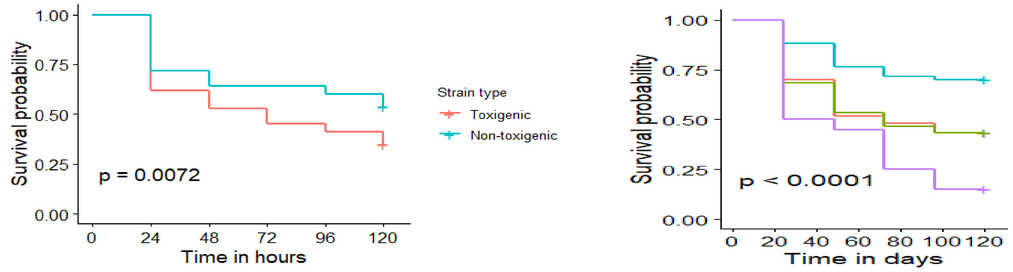

Figure 5. Effect of CDI treatment on larvae survival (n=40)

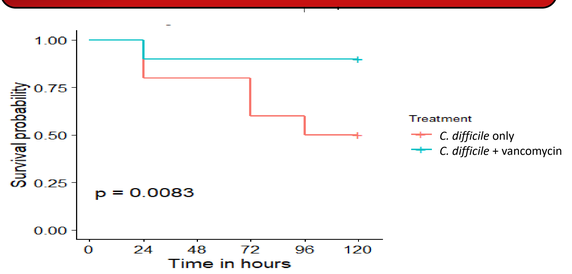

Figure 6. Larvae survival (infected vs control)

CONCLUSIONS

- ***G. mellonella* larvae can be utilized as a pre-clinical model to study the effect of antibiotic treatment**
- **This high-throughput model will be used for future pharmacology studies investigating pharmacokinetics and pharmacodynamics of antibiotics in development for CDI**

REFERENCES

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