An Invertebrate Model to Study Gut Microbiome Dysbiosis

UNIVERSITY of HOUSTON COLLEGE OF PHARMACY

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REVISED ABSTRACT

Background. Antimicrobials disrupt the gut microbiota by reducing gut microbiome diversity and quantity. *Galleria mellonella* provides an invertebrate model that is inexpensive, easy to maintain, and does not require specialized equipment. This study investigated the feasibility of using *G. mellonella* as an *in vivo* model to evaluate the effect of different antimicrobials on gut microbiota.

Methods. To determine baseline gut microbiota composition, the gut contents of *G. mellonella* were extracted and genomic DNA underwent shotgun meta-genomic sequencing. To determine the effect of infection and antimicrobial use, larvae were injected with ~$1x10^{5-8}$ colony forming unit (CFU) of three different strains of *methicillin-resistant Staphylococcus aureus* (MRSA) and were randomized 1:1:1 to treatment with vancomycin 20mg/kg or a natural antimicrobial, *Nigella sativa* seed oil 70 mg/kg (prebiotic), or a combination. The larvae were kept at 37°C post-infection and monitored daily for 120 hours for survival. After 48 hours, two larvae from each group were randomly selected and homogenized with PBS and plated for MRSA and *Enterococcus* CFU counts.

Results. Metagenomics analysis showed the gut microbiota composition of *G. mellonella* larvae was dominated by a subset of closely-related *Enterococcus* species. Survival was highest in the vancomycin and combination arms (90% and 75%, respectively). Mean *Enterococcus* CFU were lowest in the vancomycin arm and highest in the prebiotic arm ($8.1x10^4$ CFU and $2.0x10^6$ CFU, respectively (p = 0.014)). Mean MRSA CFU counts were highest in the vancomycin arm and lowest in combination arm ($3.4X10^6$ CFU and $3.2X10^5$ CFU, respectively (p = 0.0235)).

Conclusions. This study provides preliminary evidence to support the use of *G. mellonella* to assess the *in vivo* effect of natural and synthetic antimicrobials on the gut microbiota.

BACKGROUND

- The gut microbiota plays an important role in digestion and immunity.
- Antibiotic treatment is one of the most common causes of disruption in the gut microbiota.
- The invertebrate model *G. mellonella* (greater wax moth) 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.
- This popularity is attributed to its low cost, short life cycle, simple handing, and lack of ethical constraints.

OBJECTIVE

- To assess the use of *G. mellonella* as an infection model to study *Staphylococcus aureus* virulence
- To compare effect of different antimicrobials on *G. mellonella* survival
- To understand the effects of antimicrobials on gut microbiome

METHODS

Metagenomic analysis of *G. mellonella* larvae:
The gut contents of *G. mellonella* were extracted and genomic DNA underwent shotgun meta-genomic sequencing.

Larval inoculation and treatment:

- Larvae were injected (left proleg) with ~$1x10^{5-8}$ CFU of three different strains of MRSA, and treated with vancomycin 20 mg/kg, prebiotic 70 mg/kg, or a combination.
- The larvae were kept at 37°C post-infection and monitored daily for 120 hours.
- Larvae were assigned into the following arms and experiments were repeated in duplicate

1. Negative control (PBS only), n = 10
2. Positive control (MRSA only, three groups for the three strains), n = 30
3. Vancomycin treated group (three groups for the three strains), n = 30
4. Prebiotic treated group (three groups for the three strains), n = 30
5. Combination treated group (three groups for the three strains), n = 30

Determination of *S. aureus* and Enterococcus spp. in *G. mellonella* larvae:
Two larvae from each group were randomly selected, homogenized with PBS, and plated on nutrient agar for (MRSA) and m-Enterococcus agar (*Enterococcus*) CFU counts.

RESULTS

Figure 1. Metagenomic Analysis of *G. mellonella* Larvae

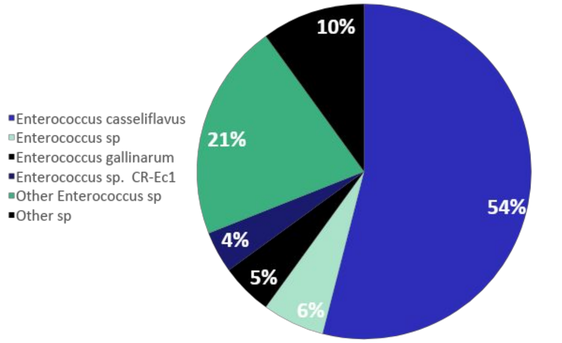


Figure 2. Larvae Overall Survival (n=300)

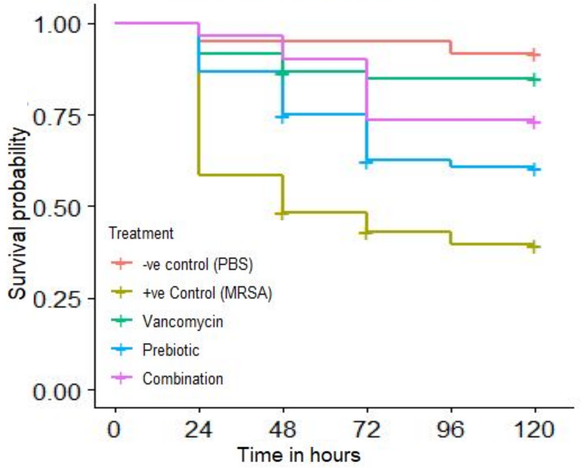


Figure 3. Mean *Enterococcus* CFU per larva

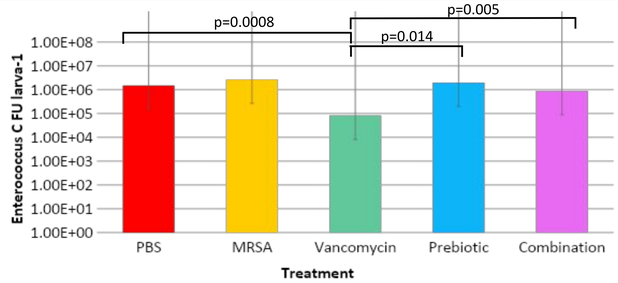
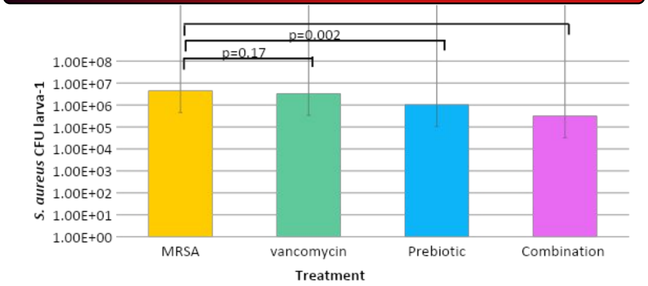


Figure 4. Mean *S. aureus* CFU per larva



CONCLUSIONS

- This study provides preliminary evidence to support the use of *G. mellonella* to assess the *in vivo* effect of the prebiotic and vancomycin on the gut microbiota.
- The prebiotic appeared to be more selective than vancomycin against MRSA while preserving the natural *Enterococcus* gut microbiota of *G. mellonella*.
- We highlight the need for larger, in-depth investigations of antimicrobial effects on the gut microbiota using *G. mellonella*.