Recent advances in *C. Elegans* as a model system for high throughput antimicrobial drug discovery

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Abstract
Multidrug resistance among pathogens has become a leading cause of health care concern since these pathogens have acquired resistance against most classes of antimicrobial drugs. Therefore there is urgent need for newer antimicrobial compounds and new treatment strategies. Unfortunately the pace of new antimicrobial drug discovery programs have slowed down due to rising cost of drug discovery coupled with the relatively unattractive markets for antimicrobial drugs compared to drugs for chronic diseases. In this background, invertebrate model organisms such as the nematode Caenorhabditis elegans and fruit fly Drosophila melanogaster are finding increasing use as models to study host-pathogen relationship and high throughput antimicrobial drug discovery. The focus of this review will be to give a brief outline of the *C. elegans* model for high throughput antimicrobial drug discovery.

Keywords: *Caenorhabditis elegans*, infectious diseases, drug resistance, high throughput screening, drug discovery.

1. Introduction
In the past several decades, antimicrobial drugs have played an important role in the overall improvement in human lifespan. In veterinary medicine, antimicrobial drugs have led to significant improvements in the quality of animal products. However, the positive effects of these drugs have been negated by their rampant use/misuse. The incidence of "antimicrobial resistance" or non-susceptibility of microorganisms to drugs that they were previously susceptible to is rapidly increasing1,2. This phenomenon of antimicrobial resistance needs to be countered with social education on responsible antimicrobial use and also with robust antimicrobial drug discovery programs.

Traditional methods of antimicrobial drug discovery involve in vitro screening and selection of test compounds, optimizing compound structure by performing Structure Activity Relationships (SAR analysis and lastly testing the compound in an in vivo disease model3. The process of transforming the compound identified in the lab into a drug that is safe for human use is both long and expensive taking up to 15 years and costing as much as a billion dollars. Additionally, the therapeutic window of antimicrobial use is relatively short due to rapid emergence of drug resistant strains. These reasons have led to mass exodus of antimicrobial discovery programs in big pharma, turning an already bad situation worse. In recent years, academic labs and small start-ups have been at the forefront of antimicrobial drug discovery.

The free living nematode *C. elegans* has recently become a popular model organism for studying pathogenesis of many bacterial and fungal pathogens3. Key virulence factors that are involved in pathogenesis in humans are also involved in pathogenesis in the nematodes. The signalling pathways by which *C. elegans* counters pathogens are also strikingly similar to those in metazoans4,5. *C. elegans* have a relatively short lifespan, are relatively inexpensive to maintain and using them as models to study host pathogen relationship does not raise the ethical or logistical concerns that arise while working with higher model organisms such as rodents. Moreover, using *C. elegans* as a test platform allows for simultaneous assessment of both the antimicrobial efficacy of the test compound and the compound’s toxicity since if the compound is toxic the worms will still not survive even if cured of the infection. Often times, compounds that display a lot of potential based on in vitro antimicrobial assays, end up being too toxic when tested in vivo in animals.

Table 1: Lists the studies which used *C. elegans* for high throughput or semi-high throughput antimicrobial drug discovery and testing.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>Bacteria:</em></td>
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<td>Enterococcus faecalis</td>
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<td>Pseudomonas aeruginosa</td>
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<td>Staphylococcus aureus</td>
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<tr>
<td><em>Fungi:</em></td>
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<tr>
<td>Candida albicans</td>
<td>13, 14</td>
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2. Work flow of the *C. elegans* antimicrobial drug discovery platform
A typical work flow of the high throughput screening platform involving *C. elegans* is similar to as shown in Fig. 1.11. The strength of using *C. elegans* in a high throughput platform is that several tasks in the work flow can be automated, reducing the workload and at the same time improving experiment reliability. The *C. elegans glp-4(bn2);sek-1(km4) double mutant strain was used in several high throughput screening studies. The *glp-4(bn2)* mutation renders the strain incapable of producing progeny at 25°C 11 and the *sek-1(km4) mutation enhances sensitivity to various pathogens16, reducing assay time. Therefore, when the *glp-4;sek-1* strain is used, the worms can be infected with the pathogen and maintained at a temperature of 25°C which would be suitable for the growth of the pathogens. Moreover, exposure of the mutant worms to 25°C renders them sterile and therefore the assay outcome will not be complicated by the production of progeny worms.

*C. elegans* is normally maintained in the laboratory at 15°C with non-pathogenic *E. coli* as food source. After hatching from the egg, nematodes undergo four larval development stages (L1-L4) before reaching adult stage. While performing *C. elegans* infection assays, worms in their L4 stage are usually preferred since they are voracious eaters and would therefore ingest even pathogens fed to them as food.
Synchronization of L1 worms after egg preparation

Worms grown till L4 stage

Exposure of L4 worms to pathogen

Transfer infected worms to 96 well plate containing test compounds

Incubation for a few days

Survival of worms in wells containing antimicrobial “hits” (Wells B3, C10, D3, E6 and G9)

Figure 1: Work flow of a typical antimicrobial high throughput screening platform using C. elegans as a whole animal host. Survival of the worms in a well containing test compound indicates that the compound is an antimicrobial hit.
3. Conclusion

The rising incidence of antimicrobial resistance among pathogens underscores the importance of identifying new treatment strategies and new methods for drug discovery. The C. elegans model for high throughput antimicrobial screening will not completely eliminate the necessity of testing potential leads in higher model system. Rather, it would serve to focus our efforts on the most promising hits by eliminating compounds which have no antimicrobial activity or too toxic, at a very early stage in the drug discovery and screening process.

References