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Letter to the Editor Open Access

Insects Offer a Useful Invertebrate Model to Screen Antimicrobial Libraries *In Vivo*

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Abstract

This report describes an invertebrate, Locusta migratoria as an *in vivo* model to screen potential antimicrobial compounds (chemical libraries) to combat infectious diseases. Locusts were infected with 2 x 106 c.f.u. of Pseudomonas aeruginosa or Methicillin-resistant Staphylococcus aureus (MRSA) and mortality recorded at 67% and 52%, respectively within 24 h. To validate the suitability of locust model to test the efficacy of potential antimicrobials, locusts were injected with *P. aeruginosa* or MRSA, followed by injection of the gentamicin. Our results show that the group treated with gentamicin resisted the bacterial infection, while the untreated group presented high mortality. It is believed that the simple locust model described in the present study has the scope in exploring the efficacy of novel drugs (testing large chemical libraries) in microbial diseases, allowing inexpensive, rapid, and even high-throughput experimentation that has no legislative restrictions.

Dear Sir,

Despite advances in chemotherapy and supportive care, infectious diseases contribute to more than 14 million deaths, annually, suggesting the need to discover novel antimicrobial compounds [1,2]. The standard approach for antimicrobial drug discovery includes *in vitro* assays for testing putative antimicrobial compounds, followed by *in vivo* experimentation using animal models. Vertebrate models are considered physiologically relevant as to provide information regarding efficacy, routes of administration and toxicity. In addition to basic screening, *in vivo* models are critical in predicting the pharmacokinetics of potential drug-leads. We have recently proposed the use of insects such as locust as an *in vivo* model to study bacterial and parasitic infections [3-5]. In the present study we propose the use of *Locusta migratoria* as a model to screen antimicrobial compounds against *Pseudomonas aeruginosa* or Methicillin-resistant *Staphylococcus aureus* (MRSA) infections *in vivo*.

Clinical isolates of *P. aeruginosa* and MRSA were obtained and cultured at 37°C for 20 h in Luria-Bertani broth as previously described [6]. Adult locusts between 15-30 days old were divided into groups of 20 and injected with 20 µL of 2 x 106 c.f.u. of *P. aeruginosa* (group 1) or MRSA (group 2) and the mortality recorded every 24 h as described previously [3-5]. The sensitivity patterns of *P. aeruginosa* and MRSA demonstrated their susceptibility to gentamicin at 100 µg/mL, *in vitro*. To validate the potency of gentamicin and the usefulness of our in vivo model system, locusts were injected with P. aeruginosa (group 3) or MRSA (group 4) as described above. After 60 min, locusts were injected with 25 µg of gentamicin, suspended in 10 µL (to obtain 100 µg/mL; as the total locust haemolymph is ~200 µL). Treatments were carried out, daily, for three days. In control, locusts were injected with non-invasive Escherichia coli K-12 strain HB101 (group 5) or saline alone (group 6) or antibiotic alone (group 7). The experiments were performed at least three times. The data are presented as the mean ± standard error. The findings revealed that P. aeruginosa and MRSA killed 67% ± 6 and 52% ± 4 locusts respectively, within three days. In contrast, locusts injected with non-invasive K-12 or saline alone showed 6% ± 1 and 2% ± 0 mortality respectively.

When treated with gentamicin, post-bacterial injection, the results showed that locusts injected with P. aeruginosa and MRSA followed by gentamicin treatment revealed 15% ± 2 and 9% ± 3 mortality respectively, within three days. Likewise, locusts injected with antibiotic alone showed 8% ± 2 mortality.

These findings support our hypothesis that insects such as locusts can prove useful in vivo models to investigate potential drug-leads as well as testing large chemical libraries. Prior to testing in vertebrates, an in vivo insect model offer several gains with regards to expense, expertise, high-throughput experimentation, ethical acceptance and legislative adherence (2010/63/EU) to replace, reduce and refine the use of animals in research. Furthermore, considerable quantities can be used, ~50 insects can be housed in a cage, that can result in meaningful n values. Any useful leads would undoubtedly need to be tested in vertebrates to determine pharmacokinetic profiles. Although in vitro assays are routinely used to screen chemical libraries, but potential antimicrobials may be missed in the early phase of the drug discovery. In spite of in vitro effectiveness, novel molecules must be tested in vivo. As long as, pharmacokinetic and pharmacodynamics differences of promising candidate molecules in locusts and vertebrates are recognized, such insects can be valuable screening tools to forecast anti-infective effectiveness of potential antimicrobials in vivo during preclinical drug development and thus reduce the number of vertebrates required overall. Although small insects such as Drosophila melanogaster has been used extensively as models for various biological processes, locusts are particularly suitable to study infectious diseases and screen chemical libraries as they are relatively large insects that can be handled with ease, injected with significant volumes (up to 20 µL), housed in simple cages, and easily captured if escaped. Using locusts, the proposed procedures can be carried out in laboratories with basic infrastructure. These findings support researches to consider using insects as tractable models to study

infectious diseases in vivo. Our proposed model is a timely response to the wishes of the public to substitute and decrease vertebrate use in research.

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