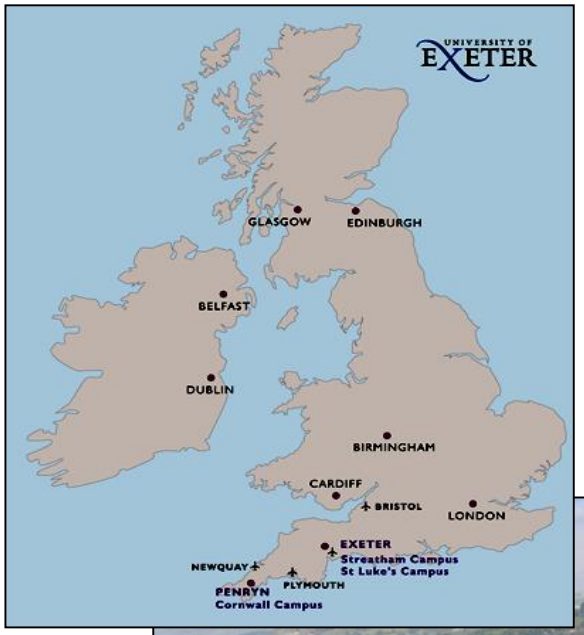
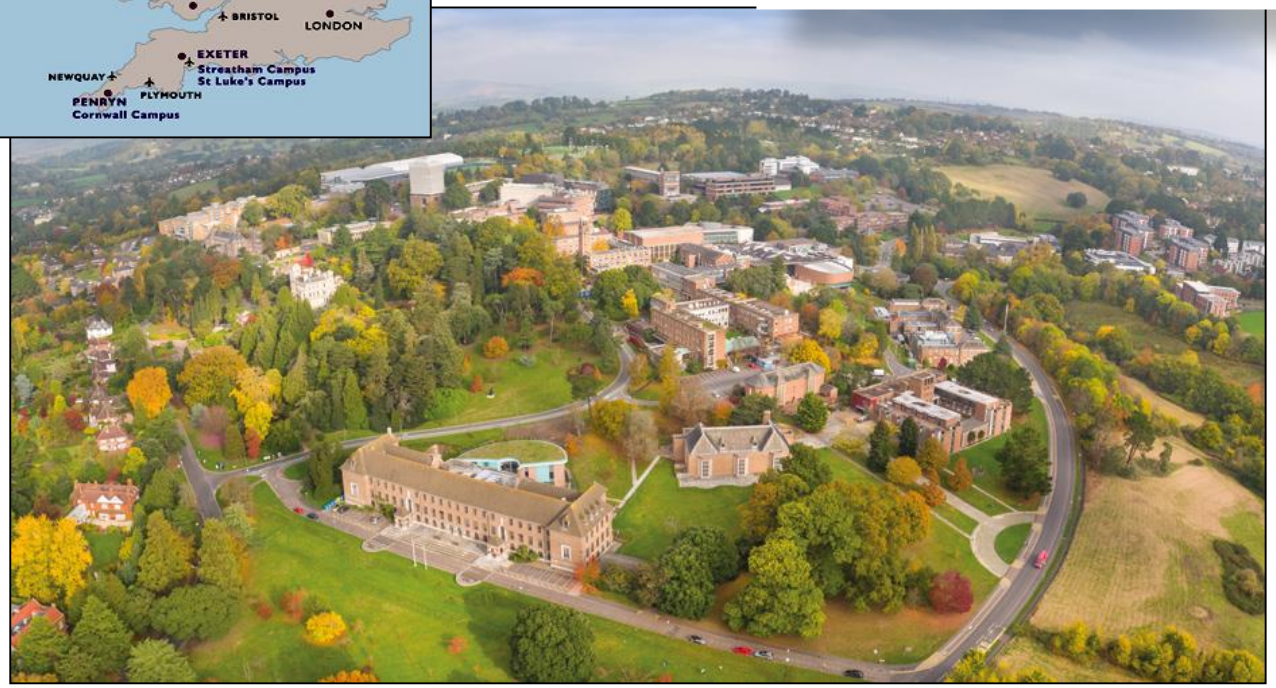
Alternative infection models



- 22000 students
- 1500 research students
- 4000 staff
- 900 academic staff

- Ranked 6th in the UK, 35th in the world

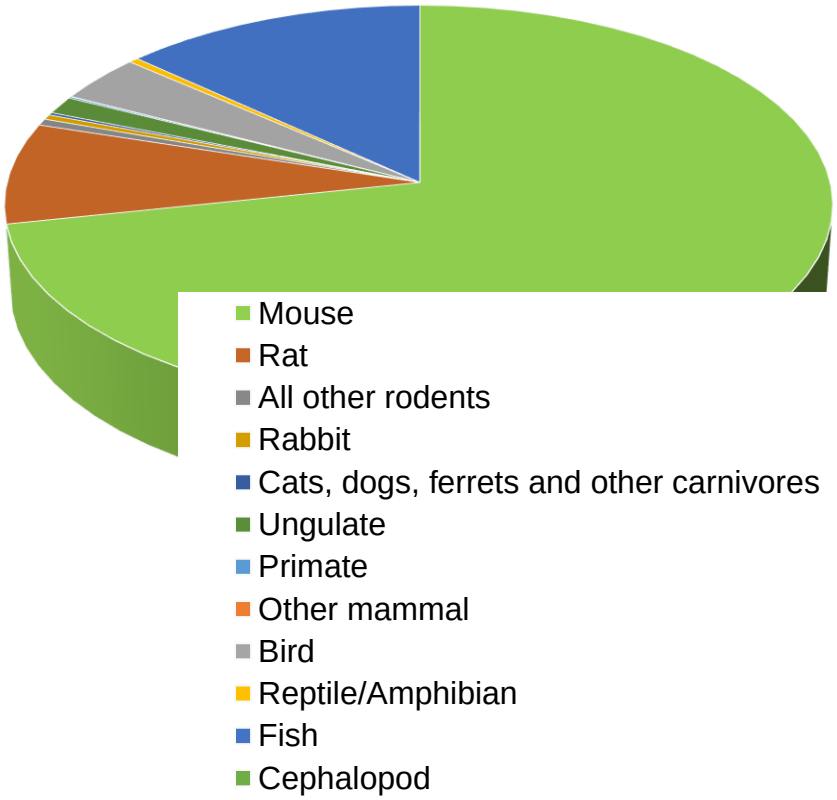
- £200m portfolio of research projects
- Fastest growing UK research university

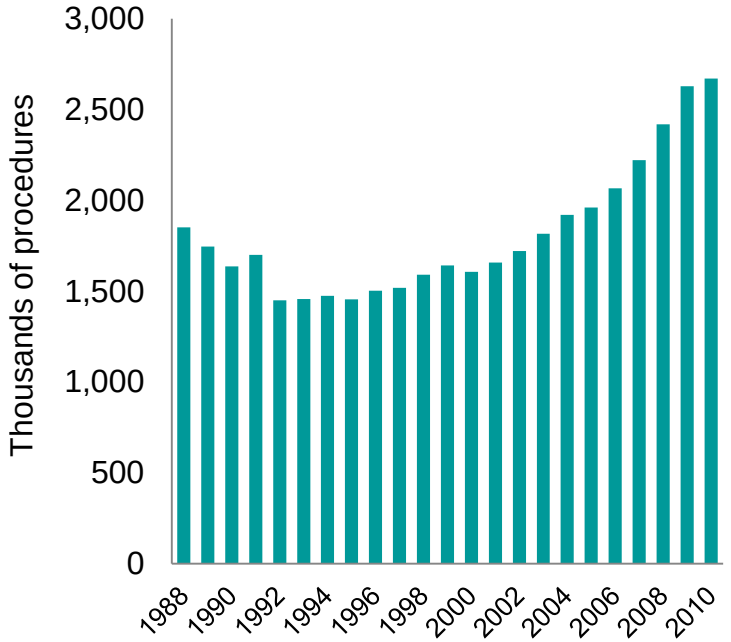


UNIVERSITY OF EXETER

UNIVERSITY OF EXETER

Animal use for experimental work in the UK

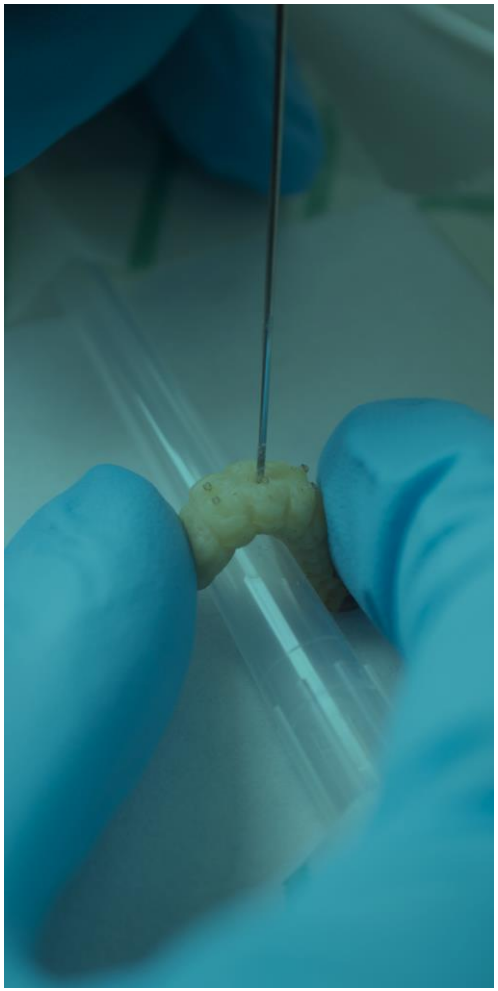




UNIVERSITY OF EXETER

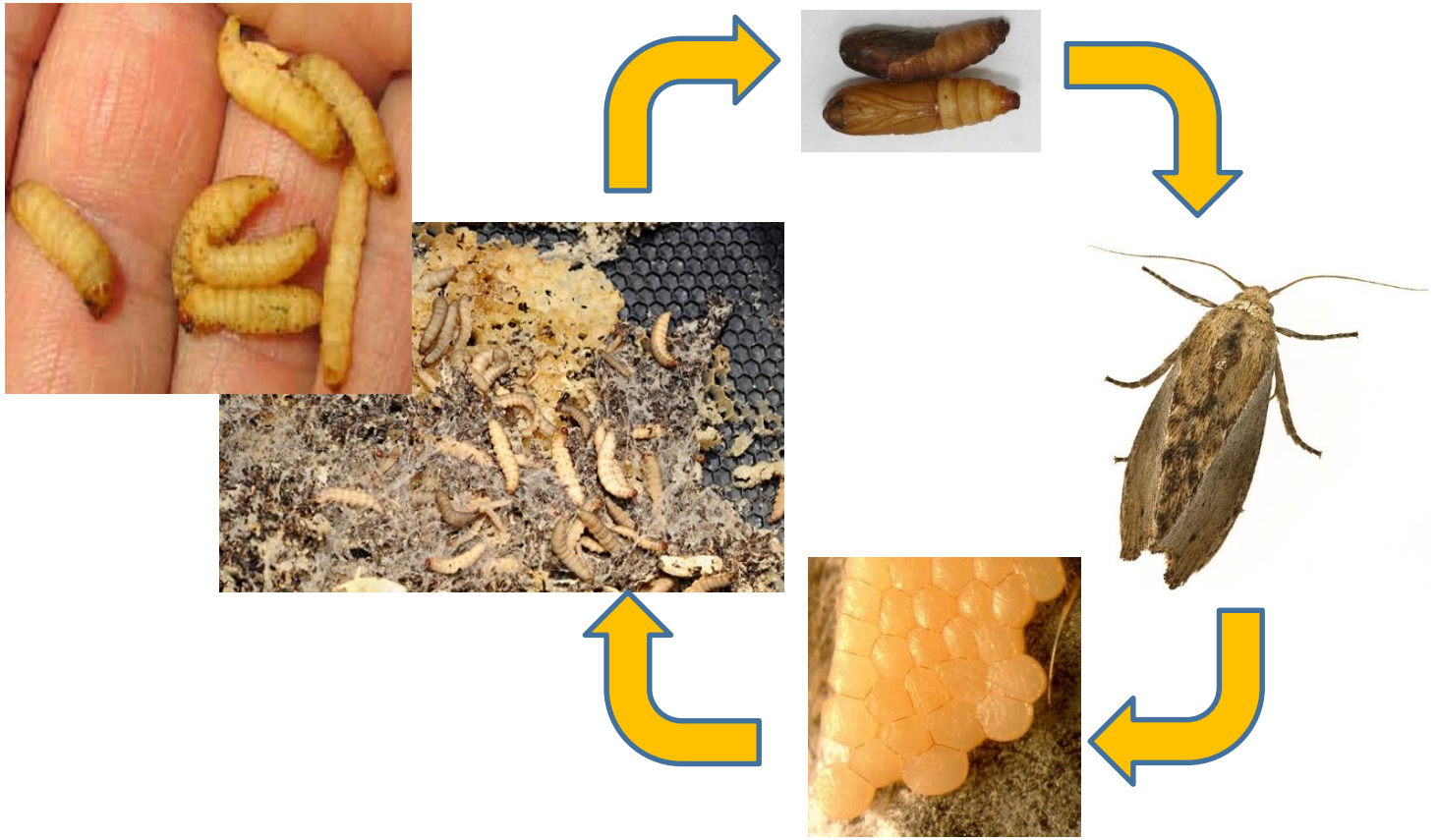
Advantages of developing non-mammalian animal models

- Ethically more acceptable
- Experiments are more cost effective
- Less labour intensive
- Can be used at an earlier project stage
- Larger experimental groups can be used providing greater power
- Can capture the complexity of a whole animal system
- Some have immune systems



UNIVERSITY OF EXETER

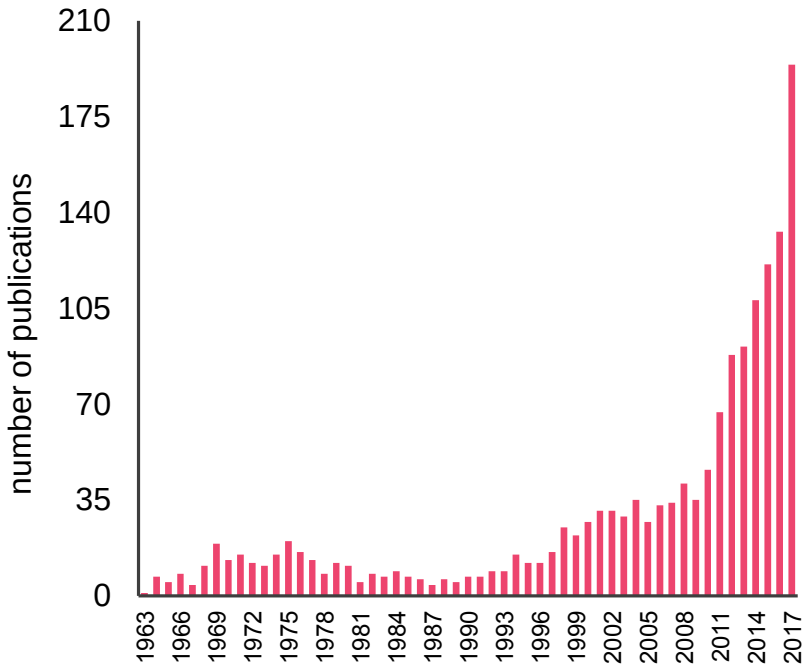
Galleria mellonella (waxmoth)



UNIVERSITY OF EXETER

G. mellonella larvae

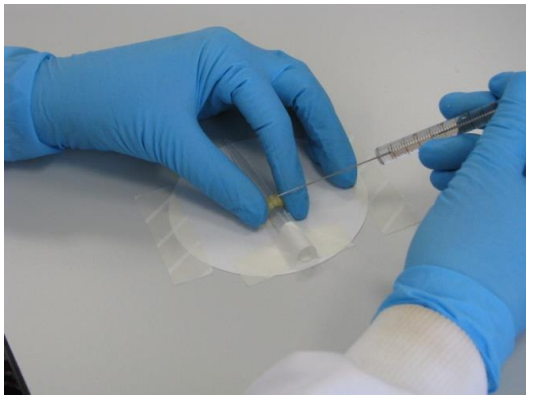
- Easy to inject with precise doses
- Incubate at 37ºC
- Possess an innate immune system
- Well developed and tested model



UNIVERSITY OF EXETER

Challenge of *G. mellonella* larvae

- Dosing via prolegs
- Disease is typically associated with a colour change
- End points either morbidity or mortality



CFU 10^6 10^4 10^2

UNIVERSITY OF EXETER

Biofilm challenge models

International Journal of Antimicrobial Agents
Volume 46, Issue 5, November 2015, Pages 538-545

Evaluation of antibiotic efficacy against infections caused by planktonic or biofilm cultures of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in *Galleria mellonella*

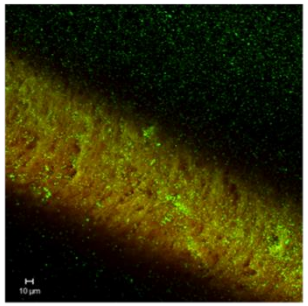
Gabriel Benthall [a, b], Rebecca E. Touzel [a], Charlotte K. Hind [a], Richard W. Titball [b], J. Mark Sutton [a], Rachael J. Thomas [b], Matthew E. Wand [a]

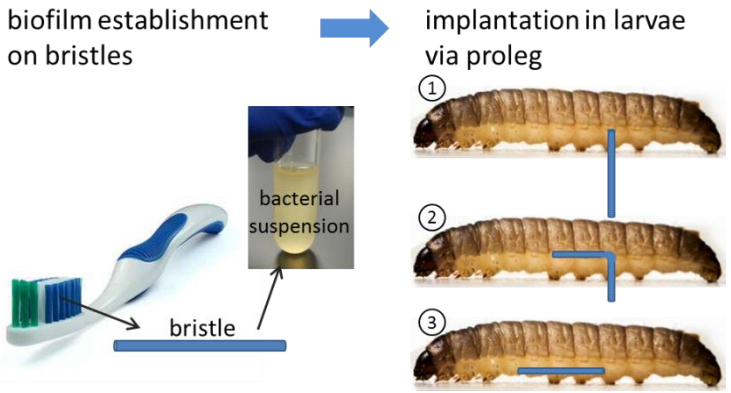
Show more

https://doi.org/10.1016/j.ijantimicag.2015.07.014

Get rights and content

E. faecalis biofilm on bristle





Lara Thieme, Institute of Infectious Diseases and Infection Control, Jena University Hospital, Germany

UNIVERSITY OF EXETER

Morbidity and mortality scoring

The *G. mellonella* Health Index Scoring System

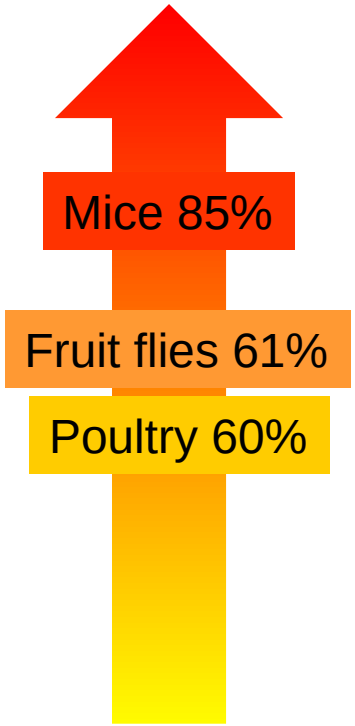
Category	Description	Score
activity	no movement	0
	minimal movement on stimulation	1
	move when stimulated	2
	move without stimulation	3
cocoon formation	no cocoon	0
	partial cocoon	0.5
	full cocoon	1
melanisation	black larvae	0
	black spots on brown larvae	1
	≥3 spots on beige larvae	2
	<3 spots on beige larvae	3
	no melanisation	4
survival	dead	0
	alive	2

Loh, J.M., et al., *Galleria mellonella* larvae as an infection model for group A streptococcus. Virulence, 2013. 4: 419-28.

UNIVERSITY OF EXETER

Humans, mice and insects

% of human protein coding genes shared with;



UNIVERSITY OF EXETER

The *G. mellonella* immune system

Cellular immunity

Hemocytes

Pro-PO system

Pro-phenoloxidase

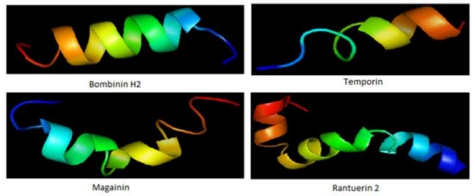
Phenoloxidase

Melanin

Humoral immunity

Pathogen recognition (TOLL, IMD, JAK/STAT, JNK)

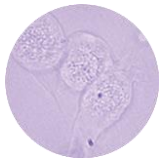
Antimicrobial peptides



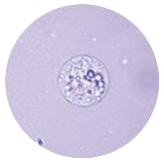
UNIVERSITY OF EXETER

Cellular immunity

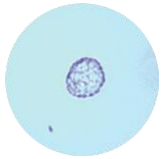
	Hemocytes	Neutrophils
Phagocytosis	Lectin-mediated	Lectin-mediated
ROS	O2−, H2O2, NO−	O2−, H2O2, NO−
Degranulation	Yes	Yes
AMPs	Peroxynectin, transferrin, lysozyme, defensin	MPO, transferrin, lysozyme, defensin
Receptors	TLRs, B-1,3-glucan, IL-IR	TLRs, B-1,3-glucan, IL-IR
Transcription factors	NFκB, IκB	NFκB, IκB
Cascades	IMD, JNK, JAK-STAT	IMD, JNK, JAK-STAT
Kinases	p38 MAPK, ERK, PKC, PKA	p38 MAPK, ERK, PKC, PKA
Neutrophil extracellular nets (NET)	NET-like structures present	NETs present



Plasmatocyte



Spherulocyte



Granular cell

Browne N, Heelan M, Kavanagh K. Virulence. 2013 4:597-603.

UNIVERSITY OF EXETER

Immuno-suppression enhances disease severity

Med Microbiol Immunol (2016) 205:333–343
DOI 10.1007/s00430-016-0450-5

ORIGINAL INVESTIGATION

Effective immunosuppression with dexamethasone phosphate in the *Galleria mellonella* larva infection model resulting in enhanced virulence of *Escherichia coli* and *Klebsiella pneumoniae*

Miquel Perez Torres[1,2] · Frances Entwistle[1] · Peter J. Coote[1]

Received: 19 November 2015 / Accepted: 11 February 2016 / Published online: 26 February 2016

Abstract The aim was to evaluate whether immunosuppression with dexamethasone 21-phosphate could be applied to the *Galleria mellonella* in vivo infection model. Characterised clinical isolates of *Escherichia coli* or *Klebsiella pneumoniae* were employed, and *G. mellonella* larvae were infected with increasing doses of each strain to investigate virulence in vivo. Virulence was then compared with larvae exposed to increasing doses of dexamethasone 21-phosphate. The effect of dexamethasone 21-phosphate on larval haemocyte phagocytosis in vitro was determined via fluorescence microscopy and a burden assay measured the growth of infecting bacteria inside the larvae. Finally, the effect of dexamethasone 21-phosphate treatment on the efficacy of ceftazidime after infection was also noted. The pathogenicity of *K. pneumoniae* or *E. coli* in *G. mellonella* larvae was dependent on high inoculum numbers such that virulence could not be attributed specifically to infection by live bacteria but also to factors associated with dead cells. Thus, for these strains, *G. mellonella* larvae do not constitute an ideal infection model. Treatment of larvae with dexamethasone 21-phosphate enhanced the lethality induced by infection with *E. coli* or *K. pneumoniae* in a dose- and inoculum size-dependent manner. This correlated with proliferation of bacteria in the larvae that could be attributed to dexamethasone inhibiting haemocyte phagocytosis and acting as an immunosuppressant. Notably, prior exposure to dexamethasone 21-phosphate reduced the efficac tazidime in vivo. In conclusion, demonstration of tive immunosuppressant regimen can improve the ity and broaden the applications of the *G. mellonel* to address key questions regarding infection.

Keywords Insect infection model · Antibacterial · Antimicrobial · Ceftazidime · Pathogenicity · Glucocorticoid anti-inflammatory

Introduction

Globally, multidrug-resistant (MDR) Gram-negati ria are a major cause of hospital-acquired infection considered an urgent public health threat [1]. Th ity of these infections are either pneumonia, blo or urinary tract infections and are associated with medical devices or surgical procedures and effect that are already debilitated or immunocom (reviewed in [2]). With increasing incidence of r to carbapenems, the antibiotics of "last-resort", on ited number of antibiotic treatments are available MDR bacteria [3] resulting in high mortality rates

In the USA, the most common MDR bacteri ated with hospital-acquired infections are *Klebsie moniae* and *Escherichia coli* [1]. MDR strains organisms initially acquired a variety of extend trum β-lactamases (ESBLs) that rendered them to cephalosporin antibiotics resulting in increase carbapenems. Subsequently, this selected for the tion of carbapenemases rendering strains resistar class of drugs also. In the UK, the first strain of *moniae* with resistance to carbapenems due to e of the *K. pneumoniae* carbapenemase 3 enzyme

✉ Peter J. Coote
pjc5@st-andrews.ac.uk

[1] Biomedical Sciences Research Complex, School of Biology, University of St Andrews, The North Haugh, St Andrews, Fife KY16 9ST, UK

[2] Present Address: Department of Microbiology, Facultad de Biología, University of Barcelona, Diagonal, 643, 08028 Barcelona, Spain

Journal of Invertebrate Pathology 98 (2008) 287–292

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/yjipa

Immunosuppressive effect of cyclosporin A on insect humoral immune response

Marta J. Fiolka *

Department of Invertebrate Immunology, Institute of Biology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland

ARTICLE INFO

Article history:

ABSTRACT

Cyclosporin A suppressed humoral immune response of *Galleria mellonella* larvae. Insects were immu- ith LPS *Pseudomonas aeruginosa* and then injected with cyclosporin A. Immunosuppressive effects pressed both, in larvae treated with cyclosporin A at the initial phase of immune response and at ctor phase of antibacterial immunity. Cyclosporin A moderately decreased lysozyme activity and antly decreased antibacterial activity peptides against *Escherichia coli*. Immunosuppressive effects sporin A were observed after immunoblotting with antibodies anti-*G. mellonella* lysozyme. Tricine GE shown that synthesis of antibacterial peptides of larvae treated with cyclosporin A was consid- nhibited. Insects of impaired immune response by cyclosporin A action lost protective immunity t bacterial pathogen *P. aeruginosa*.

PLOS ONE

Myriocin Significantly Increases the Mortality of a Non-Mammalian Model Host during *Candida* Pathogenesis

Nadja Rodrigues de Melo[1], Ahmed Abdrahman[2], Carolyn Greig[2], Krishnendu Mukherjee[3], Catherine Thornton[1], Norman A. Ratcliffe[2,4], Andreas Vilcinskas[3], Tariq M. Butt[2]*

1 College of Medicine, Swansea University, Singleton Park, Swansea, United Kingdom, 2 Department of Biosciences, College of Science, Swansea University, Singleton Park, Swansea, United Kingdom, 3 Institut für Phytopathologie und Angewandte Zoologie, Abteilung Angewandte Entomologie, Gießen, Germany, 4 Department of Biological Sciences, Universidade Federal Fluminense, Rio de Janeiro, Brazil

Abstract

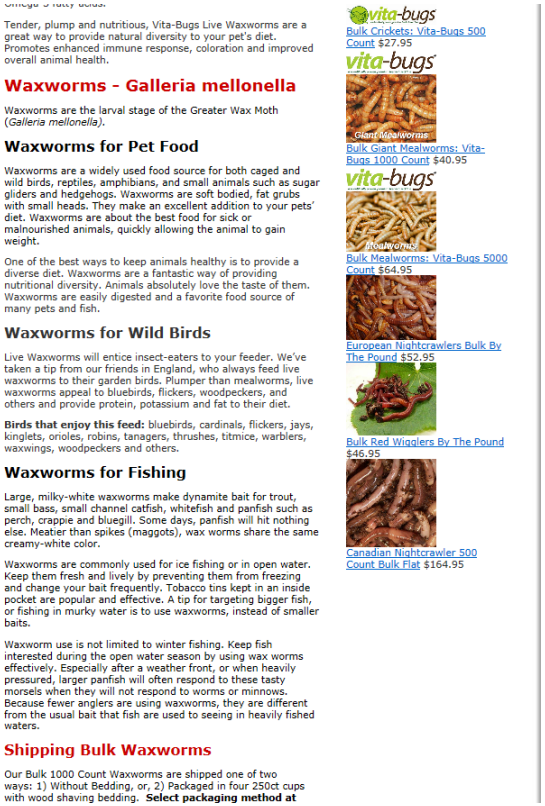
Candida albicans is a major human pathogen whose treatment is challenging due to antifungal drug toxicity, drug resistance and paucity of antifungal agents available. Myrocin (MYR) inhibits sphingosine synthesis, a precursor of sphingolipids, an important cell membrane and signaling molecule component. MYR also has dual immune suppressive and antifungal properties, potentially modulating mammalian immunity and simultaneously reducing fungal infection risk. Wax moth (*Galleria mellonella*) larvae, alternatives to mice, were used to establish if MYR suppressed insect immunity and increased survival of *C. albicans*-infected insects. MYR effects were studied *in vivo* and *in vitro*, and compared alone and combined with those of approved antifungal drugs, fluconazole (FLC) and amphotericin B (AMPH). Insect immune defenses failed to inhibit *C. albicans* with high mortalities. In insects pretreated with the drug followed by *C. albicans* inoculation, MYR+*C. albicans* significantly increased mortality to 93% from 67% with *C. albicans* alone 48 h post-infection whilst AMPH+*C. albicans* and FLC+*C. albicans* only showed 26% and 0% mortalities, respectively. MYR combinations with other antifungal drugs *in vivo* also enhanced larval mortalities, contrasting the synergistic antifungal effect of the MYR+AMPH combination *in vitro*. MYR treatment influenced immunity and stress management gene expression during *C. albicans* pathogenesis, modulating transcripts putatively associated with signal transduction/regulation of cytokines, I-kappaB kinase/NF-kappaB cascade, G-protein coupled receptor and inflammation. In contrast, all stress management gene expression was down-regulated in FLC and AMPH pretreated *C. albicans* -infected insects. Results are discussed with their implications for clinical use of MYR to treat sphingolipid-associated disorders.

Citation: Melo NRd, Abdrahman A, Greig C, Mukherjee K, Thornton C, et al. (2013) Myriocin Significantly Increases the Mortality of a Non-Mammalian Model Host

UNIVERSITY OF EXETER

Disadvantages of *Galleria mellonella*

- do not have an adaptive immune system
- larvae purchased as pet food are variable in age, size and health status
- antibiotics and hormones are commonly used in feedstuffs
- microbial flora on the surface and within larvae can result in different results from replicate experiments



Tender, plump and nutritious, Vita-Bugs Live Waxworms are a great way to provide natural diversity to your pet's diet. Promotes enhanced immune response, coloration and improved overall animal health.

Waxworms - Galleria mellonella

Waxworms are the larval stage of the Greater Wax Moth (*Galleria mellonella*).

Waxworms for Pet Food

Waxworms are a widely used food source for both caged and wild birds, reptiles, amphibians, and small animals such as sugar gliders and hedgehogs. Waxworms are soft bodied, fat grubs with small heads. They make an excellent addition to your pets' diet. Waxworms are about the best food for sick or malnourished animals, quickly allowing the animal to gain weight.

One of the best ways to keep animals healthy is to provide a diverse diet. Waxworms are a fantastic way of providing nutritional diversity. Animals absolutely love the taste of them. Waxworms are easily digested and a favorite food source of many pets and fish.

Waxworms for Wild Birds

Live Waxworms will entice insect-eaters to your feeder. We've taken a tip from our friends in England, who always feed live waxworms to their garden birds. Plumper than mealworms, live waxworms appeal to bluebirds, flickers, woodpeckers, and others and provide protein, potassium and fat to their diet.

Birds that enjoy this feed: bluebirds, cardinals, flickers, jays, kinglets, orioles, robins, tanagers, thrushes, titmice, warblers, waxwings, woodpeckers and others.

Waxworms for Fishing

Large, milky-white waxworms make dynamite bait for trout, small bass, small channel catfish, whitefish and panfish such as perch, crappie and bluegill. Some days, panfish will hit nothing else. Meatier than spikes (maggots), wax worms share the same creamy-white color.

Waxworms are commonly used for ice fishing or in open water. Keep them fresh and lively by preventing them from freezing and change your bait frequently. Tobacco tins kept in an inside pocket are popular and effective. A tip for targeting bigger fish, or fishing in murky water is to use waxworms, instead of smaller baits.

Waxworm use is not limited to winter fishing. Keep fish interested during the open water season by using wax worms effectively. Especially after a weather front, or when heavily pressured, larger panfish will often respond to these tasty morsels when they will not respond to worms or minnows. Because fewer anglers are using waxworms, they are different from the usual bait that fish are used to seeing in heavily fished waters.

Shipping Bulk Waxworms

Our Bulk 1000 Count Waxworms are shipped one of two ways: 1) Without Bedding, or, 2) Packaged in four 250ct cups with wood shaving bedding. **Select packaging method at**

vita-bugs

Bulk Crickets: Vita-Bugs 500 Count $27.95

vita-bugs

Bulk Giant Mealworms: Vita-Bugs 1000 Count $40.95

vita-bugs

Bulk Mealworms: Vita-Bugs 5000 Count $64.95

European Nightcrawlers Bulk By The Pound $52.95

Bulk Red Wigglers By The Pound $46.95

Canadian Nightcrawler 500 Count Bulk Flat $164.95

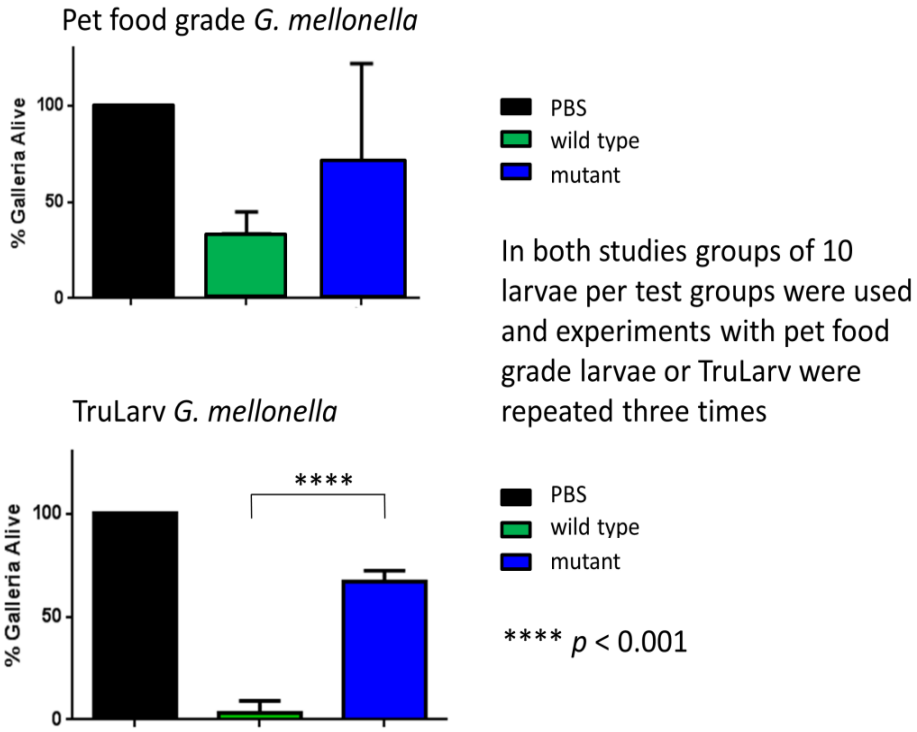
UNIVERSITY OF EXETER

Sources of larvae

- Research grade larvae (TruLarv™)
  - Genetically defined breeding colony
  - Age and weight matched
  - Raised without addition of antibiotics or hormones
  - Surface decontaminated
  - Batch documentation and quality control

UNIVERSITY OF EXETER

Sources of larvae



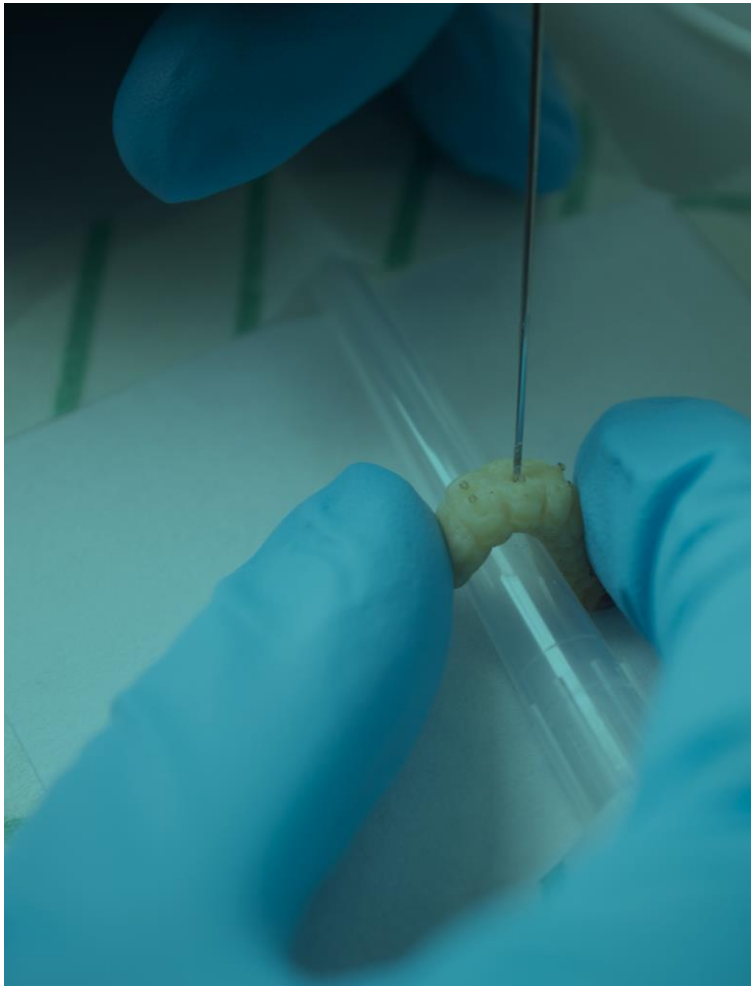
- Compare wild type and *mutT* mutants of *V. parahaemolyticus*
- Cannot discriminate using pet food larvae (8 separate studies)
- Can clearly see the difference using research grade larvae

Wagley S et al., Virulence. 2018 9:197-207

UNIVERSITY OF EXETER

Applications

- Infection models to study virulence
- Antimicrobial Drug screening
- Chemical toxicity testing



UNIVERSITY OF EXETER

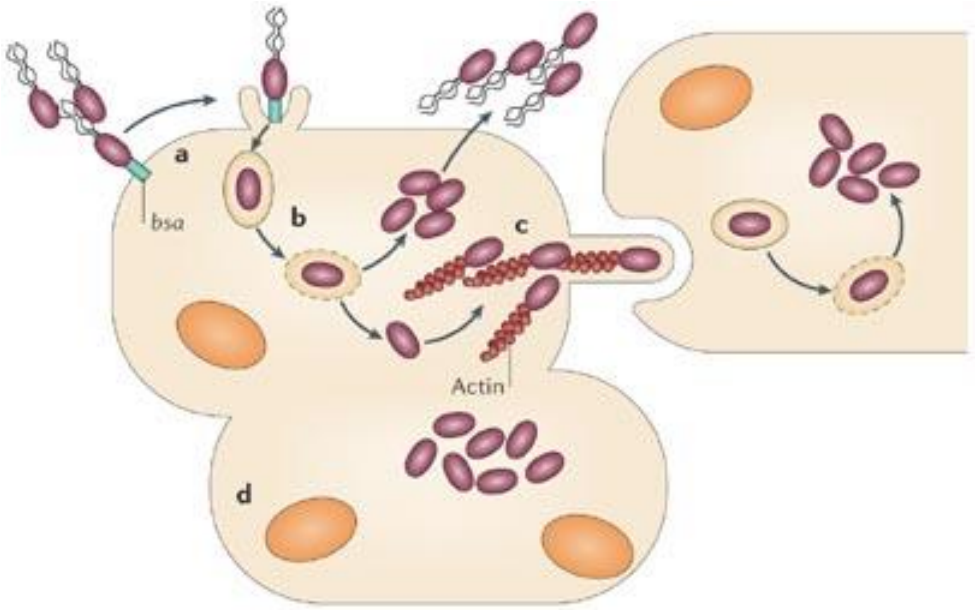
Infection models to study virulence

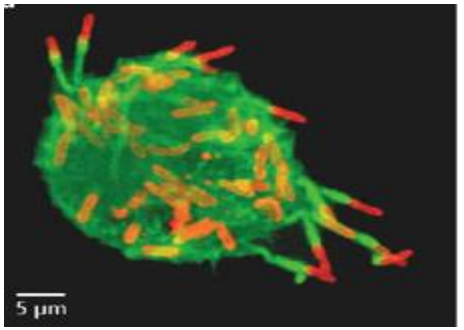
Burkholderia pseudomallei

- Etiological agent of melioidosis
- Estimated global incidence 165,000 cases (42,000 deaths)
- The most frequent cause of community acquired septicaemia in N. Thailand
- *B. thailandensis* is a low-virulence relative

UNIVERSITY OF EXETER

Intracellular lifestyles of *B. pseudomallei and B. thailandensis*

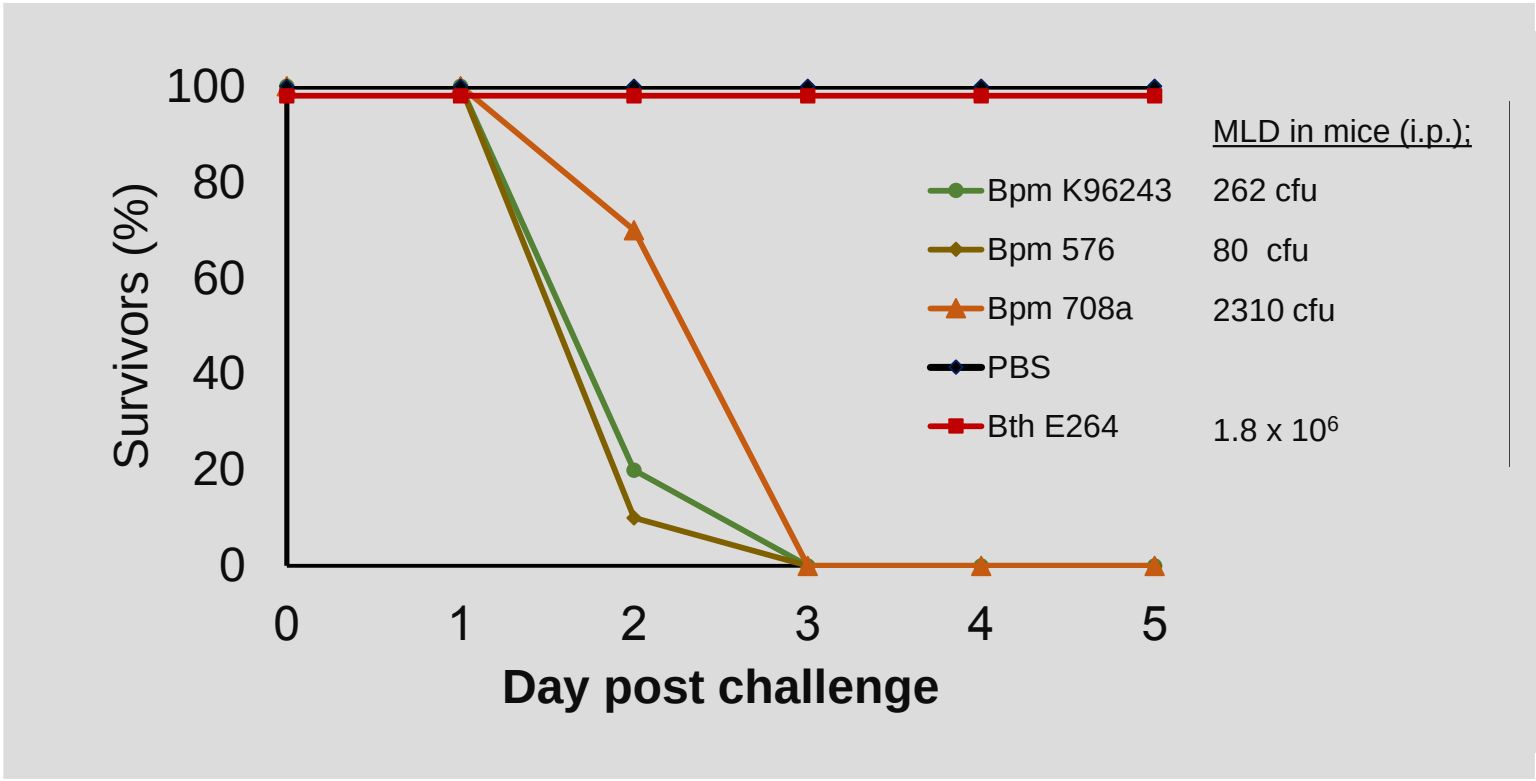




Wiersinga WJ *et al. Nature Reviews Microbiology* **4**, 272–282 (April 2006) | doi:10.1038/nrmicro1385

UNIVERSITY OF EXETER

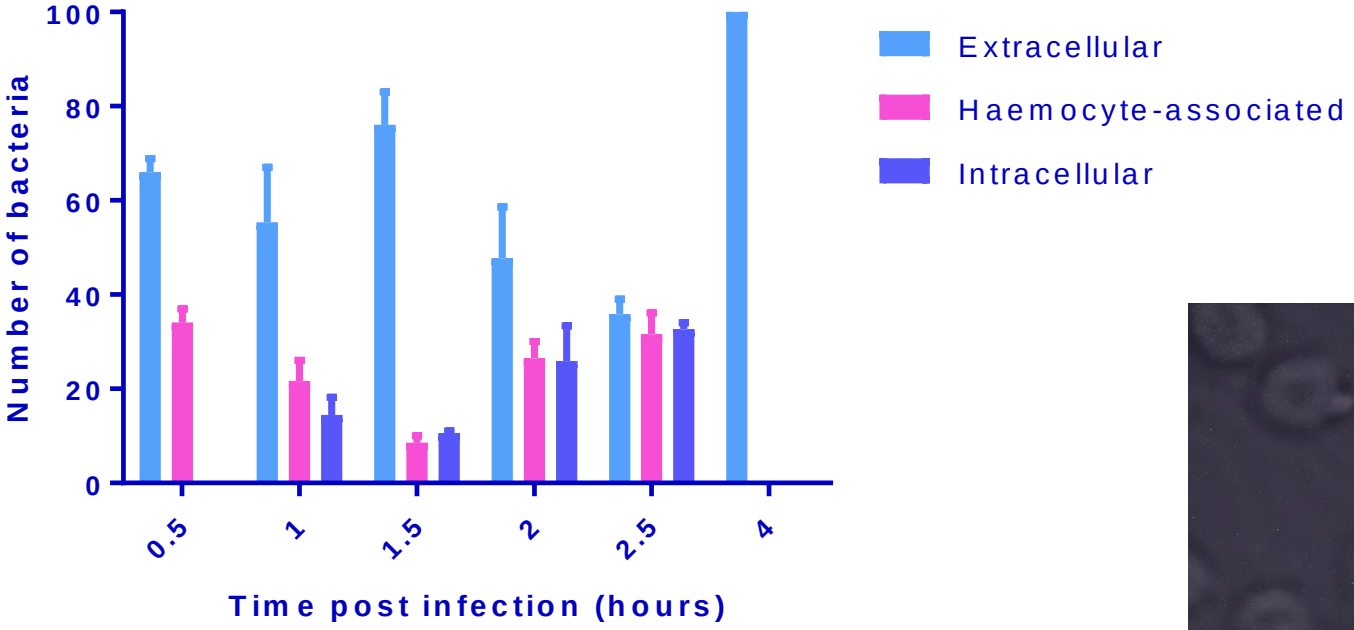
G. mellonella reveals differences in virulence

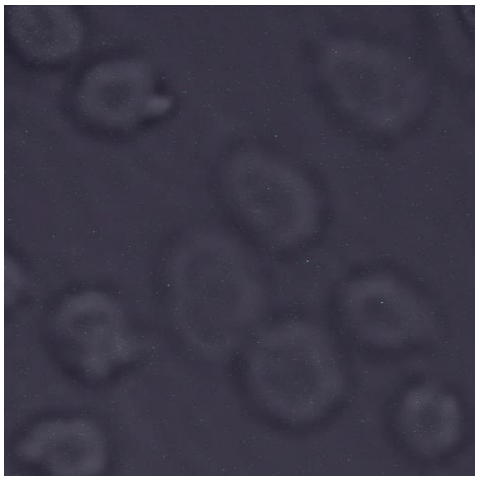


Challenge with 10 cfu

UNIVERSITY OF EXETER

Burkholderia are able to invade hemocytes

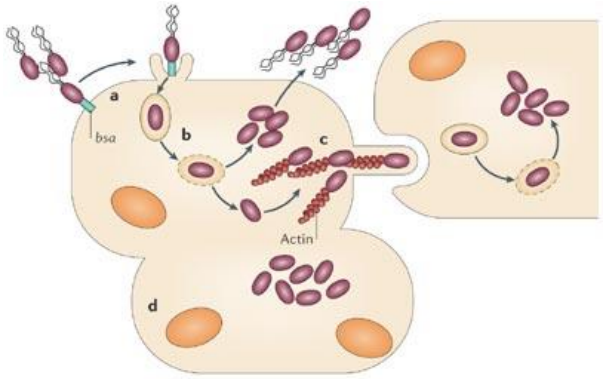




Thomas *et al.* 2013 *Galleria mellonella* as a model system to test the pharmacokinetics and efficacy of antibiotics against *Burkholderia pseudomallei*. *Int. J. Anti. Agents. 41(4):* 330-336

UNIVERSITY OF EXETER

B. pseudomallei treA mutant is attenuated in *G. mellonella* and in mice



Virulence

Virulence

ISSN: 2150-5594 (Print) 2150-5608 (Online) Journal homepage: http://www.tandfonline.com/loi/kvir20

Trehalase plays a role in macrophage colonization and virulence of Burkholderia pseudomallei in insect and mammalian hosts

Muthita Vanaporn, Mitali Sarkar-Tyson, Andrea Kovacs-Simon, Philip M. Ireland, Pornpan Pumirat, Sunee Korbsrisate, Richard W. Titball & Aaron Butt

To cite this article: Muthita Vanaporn, Mitali Sarkar-Tyson, Andrea Kovacs-Simon, Philip M. Ireland, Pornpan Pumirat, Sunee Korbsrisate, Richard W. Titball & Aaron Butt (2016): Trehalase plays a role in macrophage colonization and virulence of Burkholderia pseudomallei in insect and mammalian hosts, Virulence, DOI: 10.1080/21505594.2016.1199316

To link to this article: http://dx.doi.org/10.1080/21505594.2016.1199316

View supplementary material

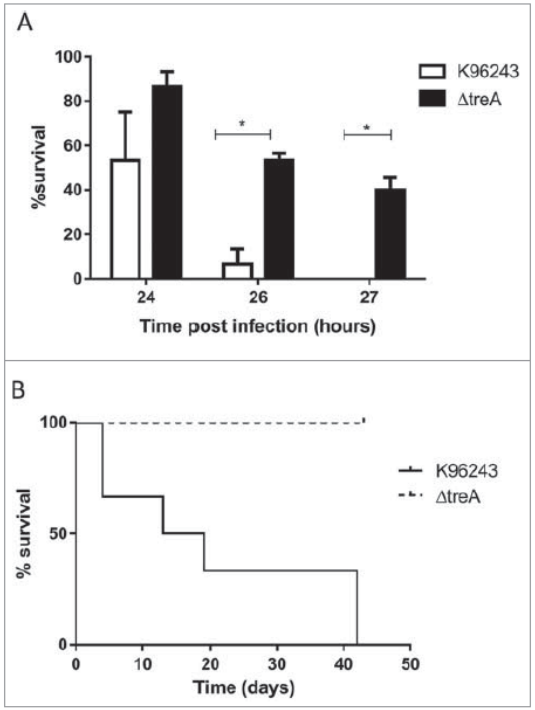


Figure 6. Virulence of *B. pseudomallei* wild type or Δ*treA* mutant in *G. mellonella* larvae or in mice. (A) Groups of 10 *G. mellonella* larvae were challenged with 10^3 CFU of *B. pseudomallei* K96243 or the *ΔtreA* mutant. Values represent the mean from 3 independent experiments. Error bars show SEM. * = $p < 0.05$ following 2 way Anova, Sidak's multiple comparisons test. (B) Groups of 6 BALB/c mice challenged via i.p. route with 3.4×10^4 CFU of *B. pseudomallei* K96243 (solid line) or 6.7×10^4 CFU of the *ΔtreA* mutant (dotted line).

UNIVERSITY OF EXETER

G. mellonella bacterial infection models reported

Acinetobacter baumannii	(Peleg et al. 2009a; Hornsey and Wareham 2011)
Acinetobacter indicus	(Klotz et al. 2017)
Acinetobacter nosocomialis	(Chusri et al. 2014)
Acinetobacter pittii	(Chusri et al. 2014)
Actinobacillus pleuropneumoniae	(Pereira et al. 2015)
Bacillus anthracis	(Blower et al. 2017)
Bacillus cereus	(Salamitou et al. 2000; Fedhila et al. 2006)
Brucella melitensis	(Sprynski et al. 2014)
Brucella suis	(Sprynski et al. 2014)
Burkholderia ambifaria	(Vial et al. 2010)
Burkholderia cepacia complex	(Seed and Dennis 2008; Tegos et al. 2012)
Burkholderia cenocepacia	(Uehlinger et al. 2009; Schwager et al. 2013)
Burkholderia mallei	(Schell et al. 2008)
Burkholderia multivorans	(Silva et al. 2011; Schmerk and Valvano 2013)
Burkholderia pseudomallei	(Schell et al. 2008; Wand et al. 2011)
Campylobacter jejuni	(Champion et al. 2010b; Askoura and Stintzi 2017)
Clostridium difficile	(Nale et al. 2016)
Cronobacter sakazakii	(Abbasifar et al. 2014)
Coxiella burnettii	(Norville et al. 2014; Martinez et al. 2016)
Enterobacter cloacae	(Betts et al. 2014a)
Enterococcus faecalis	(Gaspar et al. 2009)
Enterococcus faecium	(Lebreton et al. 2011)
Escherichia coli	(Dudziak and Jozwik 1969)
Escherichia coli ExPEC	(Dudziak and Jozwik 1969; Nathan 2014
Escherichia coli UPEC	(Alghoribi et al. 2014)
Escherichia coli EPEC	(Leuko and Raivio 2012; Younas et al. 2016)
Escherichia coli EAEG	(Jonsson et al. 2016)
Escherichia coli EHEC	(Morgan et al. 2014)
Francisella philomiragia	(Propst et al. 2016)
Francisella tularensis	(Aperis et al. 2007)
Helicobacter pylori	(Giannouli et al. 2014)
Klebsiella pneumoniae	(Insua et al. 2013; Benthall et al. 2015)
Legionella pneumophila	(Harding et al. 2012; Harding et al. 2013)
Listeria monocytogenes	(Mukherjee et al. 2010; Browne and Kavanagh 2013)
Proteus mirabilis	(Howery et al. 2016)
Pseudomonas aeruginosa	(Kropinski and Chadwick 1975; Jander et al. 2000)
Salmonella enterica Typhimurium	(Kurstak and Vega 1968; Viegas et al. 2013)
Serratia marcescens	(Chadwick et al. 1990; Gruber et al. 2015)
Shigella sonnei	(Mahmoud et al. 2016)
Staphylococcus aureus (including MRSA)	(Peleg et al. 2009b; Boakes et al. 2016)
Stenotrophomonas maltophilia	(Nicoletti et al. 2011; Betts et al. 2014b)
Streptococcus mutans	(Abranches et al. 2011; Bitoun et al. 2012)
Streptococcus pneumoniae	(Evans and Rozen 2012; Loh et al. 2013)
Streptococcus pyogenes	(Olsen et al. 2011)
Streptococcus suis	(Velikova et al. 2016)
Group A streptococci	(Olsen et al. 2011; Loh et al. 2013)
Vibrio anguillarum	(McMillan et al. 2015)
Vibrio cholera	(Nuidate et al. 2016)
Yersinia entercolitica	(Fuchs et al. 2008)
Yersinia pestis	(Erickson et al. 2011; Ford et al. 2014)
Yersinia pseudotuberculosis	(Champion et al. 2009; Strong et al. 2011)

Notable exception; Neisseria

UNIVERSITY OF EXETER

G. mellonella fungal infection models reported

Aspergillus fumigatus	(Jackson, Higgins et al. 2009, Geissel, Penka et al. 2017)
Candida albicans	(Cotter, Doyle et al. 2000, Brennan, Thomas et al. 2002)
Candida auris	(Borman, Szekely et al. 2016)
Candida dubliniensis	(Junqueira, Fuchs et al. 2011)
Candida glabrata	(Junqueira, Fuchs et al. 2011, Borghi, Andreoni et al. 2014)
Candida kefyr	(Junqueira, Fuchs et al. 2011)
Candida krusei	(Junqueira, Fuchs et al. 2011)
Candida lusitaniae	(Junqueira, Fuchs et al. 2011)
Candida novergensis	(Junqueira, Fuchs et al. 2011)
Candida parapsilosis complex	(Gago, Garcia-Rodas et al. 2014, Souza, Fuchs et al. 2015)
Candida tropicalis	(Mesa-Arango, Forastiero et al. 2013, Moralez, Perini et al. 2016)
Cryptococcus deneoformans	(Gago, Serrano et al. 2017)
Cryptococcus gattii	(Firacative, Duan et al. 2014)
Cryptococcus neoformans	(Mylonakis, Moreno et al. 2005)
Paracoccidioides brasiliensis	(de Lacorte Singulani, Scorzoni et al. 2016)
Paracoccidioides lutzii	(de Lacorte Singulani, Scorzoni et al. 2016)
Scedosporium boydii	(Rollin-Pinheiro, de Meirelles et al. 2017)
Scedosporium aurantiacum	(Rollin-Pinheiro, de Meirelles et al. 2017)
Sporothrix brasiliensis	(Clavijo-Giraldo, Matinez-Alvarez et al. 2016)
Sporothrix schenckii	(Clavijo-Giraldo, Matinez-Alvarez et al. 2016)
Trichosporon asahii	(Marine, Bom et al. 2015)
Trichosporon asteroids	(Marine, Bom et al. 2015)
Trichosporon inkin	(Marine, Bom et al. 2015)

Notable exception; *Pneumoncystis murina*

UNIVERSITY OF EXETER

Antimicrobial drug screening

Antibiotic resistance of *B. pseudomallei*

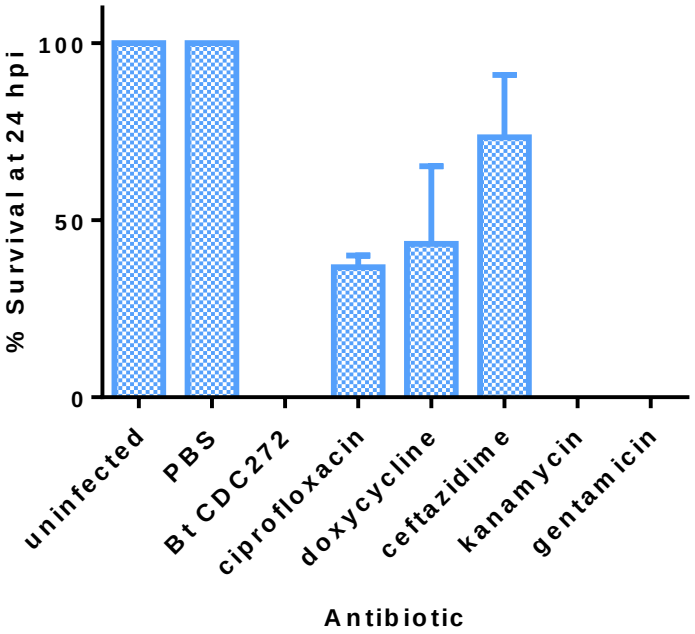
- Resistant to many antibiotics
  - many penicillins
  - macrolides
  - aminoglycosides
  - early cephalosporins
  - polymyxins
  - rifamycins
- Usually treated with
  - ceftazidime
  - (doxycline)

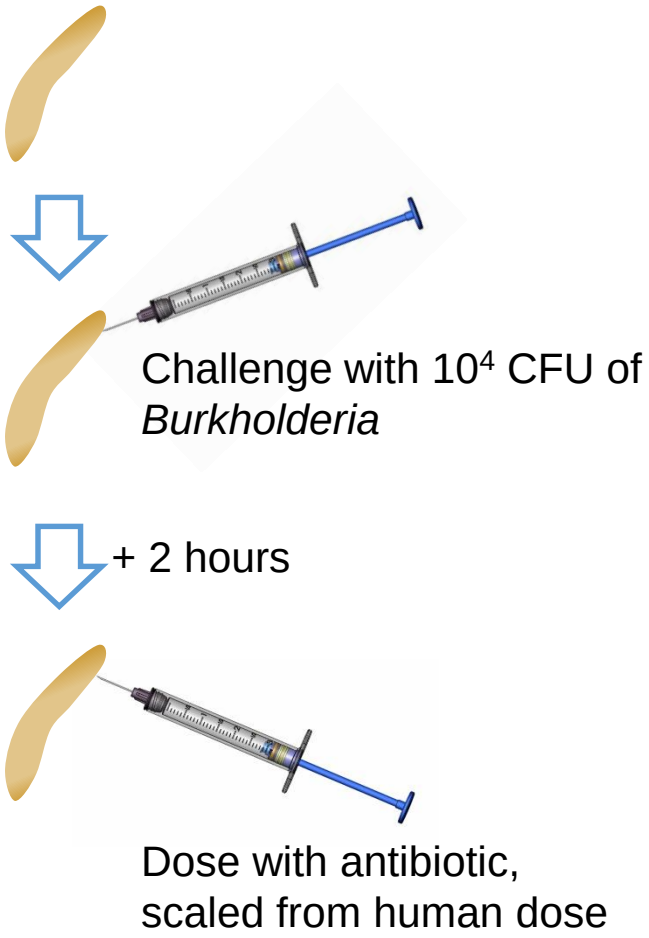
UNIVERSITY OF EXETER

Can clinically relevant antibiotics rescue *G. mellonella*?

Antibiotic	Sensitive/Resistant	Lipid Soluble	Hypothesis for activity *in vivo*
ceftazidime (120mg/kg)	Sensitive	Yes	Yes
doxycycline (80mg/kg)	Sensitive	Yes	Yes
kanamycin (15mg/kg)	Sensitive	No	No
gentamycin (3mg/kg)	Resistant	No	No
ciprofloxacin (20mg/kg)	Sensitive	Yes	Yes

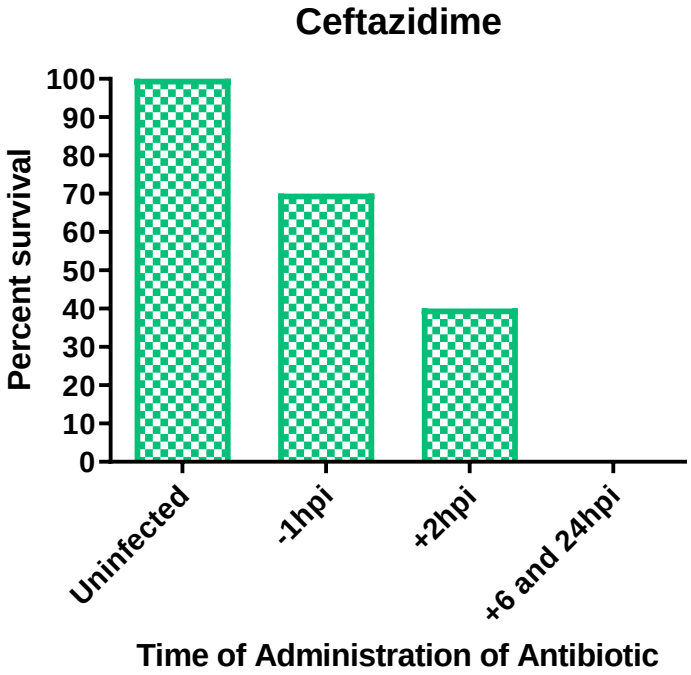
G. mellonella predicts the efficacy of clinically relevant antimicrobials





UNIVERSITY OF EXETER

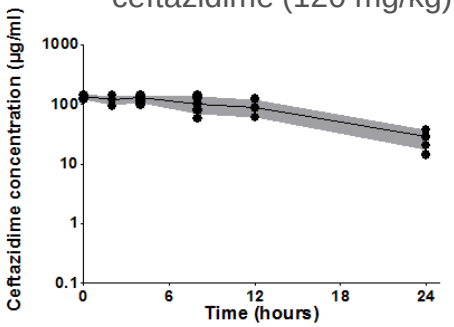
Timing of antibiotic dosing affects outcome



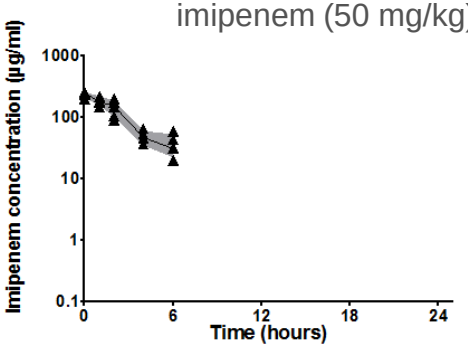
UNIVERSITY OF EXETER

Antibiotic clearance from *G. mellonella* hemolymph

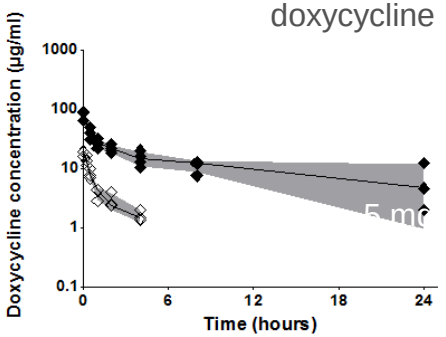
ceftazidime (120 mg/kg)



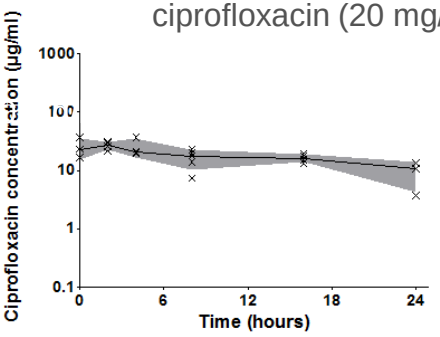
imipenem (50 mg/kg);



doxycycline



ciprofloxacin (20 mg/kg)



Thomas *et al.* 2013 *Galleria mellonella* as a model system to test the pharmacokinetics and efficacy of antibiotics against *Burkholderia pseudomallei*. *Int. J. Anti. Agents. 41(4):* 330-336

Antibiotics were administered to the larvae at doses scaled from the human dose on a weight basis and the concentration of antibiotic in haemolymph determined. Graph shows median and each replicate.

UNIVERSITY OF EXETER

Antibiotic pharmacokinetics in humans and in *G. mellonella*

Human

Antibiotic	Dose (mg/kg/day)	Dose	Cmax (ug/ml)	AUC (h*ug/mL)	Half life (hours)
Ceftazidime	120	6 g day^{-1} iv	89.6	1496	11.89
Ciprofloxacin	20	750 mg day^{-1} po	4.3	31.6	4
Doxycycline	3.3	200 mg day^{-1} po	2.6-5.9	40-123	13-25

G. mellonella

Antibiotic	Cmax (ug/ml)	AUC (h*ug/mL)	Half life (hours)
Ceftazidime	134.18	2047.85	8.15
Ciprofloxacin	26.98	522.25	10.42
Doxycycline	80.48	299.59	17.04

UNIVERSITY OF EXETER

Existing antimicrobials tested in *G. mellonella*

drug class	drug	pathogen	reference
ANTIBACTERIAL			
Penicillins	Piperacillin	*P. aeruginosa*	(Hill et al. 2014; Krezdorn et al. 2014; Adamson et al. 2015)
	Meropenem	*P. aeruginosa, Acinetobacter baumannii*	(Peleg et al. 2009; Hill et al. 2014; Krezdorn et al. 2014; Adamson et al. 2015)
	Ampicillin	*E. faecium*	(Chibebe Junior et al. 2013)
	Doripenem	*Acinetobacter baumannii*	(O'Hara et al. 2013)
	Imipenem	*B. pseudomallei*	(Thomas et al. 2013)
	Penicillin	*S. aureus (MSSA & MRSA)*	(Desbois and Coote 2011)
Cephalosporins	cefotaxime	*P. aeruginosa, Acinetobacter baumannii*	(Peleg et al. 2009; Hill et al. 2014; Krezdorn et al. 2014)
	Ceftazidime	*B. pseudomallei*	(Thomas et al. 2013)
Aminoglycosides	Amikacin	*P. aeruginosa*	(Hill et al. 2014; Krezdorn et al. 2014)
	Gentamicin	*E. faecium, Acinetobacter baumannii*	(Peleg et al. 2009; Chibebe Junior et al. 2013)
	Kanamycin	*B. pseudomallei*	(Thomas et al. 2013)
	Streptomycin	*F. tularensis*	(Aperis et al. 2007)
Polymyxins	colistin	*Stenotrophomonas maltophilia, Acinetobacter baumannii*	(Hornsey and Wareham 2011; O'Hara et al. 2013; Betts et al. 2014; Hill et al. 2014)
Glycylcylines	Tigecycline	*Stenotrophomonas maltophilia*	(Betts et al. 2014)
Rifamycins	Rifampicin	*Stenotrophomonas maltophilia*	(Betts et al. 2014)
Tetracyclines	Doxycycline	*Coxiella burnettii, B. pseudomallei*	(Thomas et al. 2013; Norville et al. 2014)
	Tetracycline	*Acinetobacter baumannii*	(Peleg et al. 2009)
Glycopeptides	Vancomycin	*S. aureus (MSSA & MRSA), Acinetobacter baumannii*	(Desbois and Coote 2011; O'Hara et al. 2013; Yang et al. 2015)
Fluoroquinolones	Ciprofloxacin	*B. pseudomallei, F. tularensis*	(Aperis et al. 2007; Thomas et al. 2013)
	Levofloxacin	*P. aeruginosa, F. tularensis*	(Aperis et al. 2007; Hill et al. 2014; Adamson et al. 2015)
Lipopeptide	Daptomycin	*S. aureus (MSSA & MRSA)*	(Desbois and Coote 2011)
Macrolide	Azithromycin	*F. tularensis*	(Ahmad et al. 2010)
ANTIFUNGAL			
Triazoles	Fluconazole	*C. albicans, C. tropicalis, Trichosporon asahii, Trichosporon asteroides and Trichosporon inkin*	(Forastiero et al. 2013; Li et al. 2013; Mesa-Arango et al. 2013; Marine et al. 2015)
	Voriconazole	*C. tropicalis, C. krusei, Trichosporon asahii, Trichosporon asteroides and Trichosporon inkin*	(Forastiero et al. 2013; Mesa-Arango et al. 2013; Scorzoni et al. 2013; Marine et al. 2015)
Polyenes	amphotericin B	*C. albicans, C. tropicalis, C. krusei, Aspergillus terreus, Cryptococcus neoformans*	(Mylonakis et al. 2005; Forastiero et al. 2013; Li et al. 2013; Mesa-Arango et al. 2013; Scorzoni et al. 2013; Blatzer et al. 2015)
Pyrimidine analogues	Flucytosine	*C. albicans, Cryptococcus neoformans*	(Mylonakis et al. 2005; Li et al. 2013)
Lipopeptide	Caspofungin	*C. krusei, C. tropicalis*	(Mesa-Arango et al. 2013; Scorzoni et al. 2013)

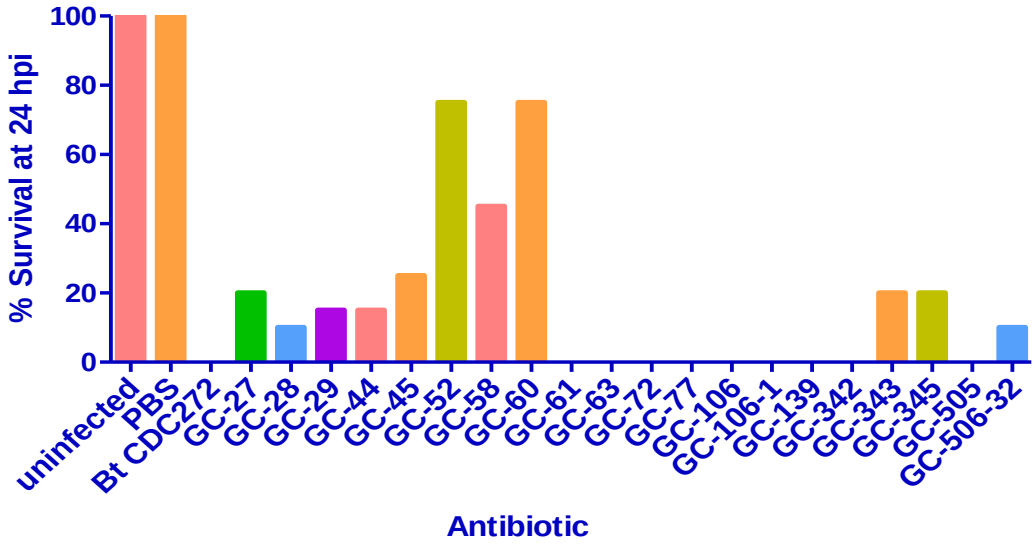
UNIVERSITY OF EXETER

Testing new therapeutics

- Combinations antibiotics (Betts et al. 2014)(Krezdorn et al. 2014) (Hornsey and Wareham 2011; Hornsey et al. 2013; O'Hara et al. 2013)
- Combinations of antifungals (Mylonakis et al. 2005) .
- Combinations of existing and novel drugs (Adamson et al. 2015) (Blatzer et al. 2015) (Farha et al. 2013)
- Bacteriophages (Seed and Dennis 2009), or antibiotics and bacteriophages (Kamal and Dennis 2015)
- Novel drugs (Antunes et al. 2012; Ross-Gillespie et al. 2014)(Koch et al. 2014) (Brackman et al. 2011) (McKenney et al. 2012)(Bastidas et al. 2012) (Mil-Homens et al. 2012) (Rowan et al. 2009; Browne et al. 2014) (Vu and Gelli 2010) (Cowen et al. 2009)
- Antimicrobial peptides (Gibreel and Upton 2013)(Dean et al. 2011) (Brown et al. 2008)
- Antimicrobial peptides (Gibreel and Upton 2013)(Dean et al. 2011) (Brown et al. 2008)
- Plant derived antimicrobials (Favre-Godal et al. 2014)
- Antimicrobial photodynamic therapy for (Chibebe Junior et al. 2013)
- Drug delivery systems (Deacon et al. 2015)(Coughlan et al. 2010)
- Reports that immunosuppressive drugs, such as myrocin, enhance the susceptibility of *G. mellonella* to infection with Candida albicans and this may allow new opportunities for the testing of antimicrobials in a clinically relevant model (de Melo et al. 2013). Similarly, immunosuppression with cyclospron A reduced resistance of *G. mellonella* to infection with P. aeruginosa (Fiolka 2008)

UNIVERSITY OF EXETER

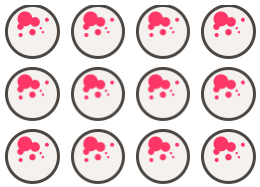
High throughput screening of novel antimicrobials



Thomas *et al.* 2013 *Galleria mellonella* as a model system to test the pharmacokinetics and efficacy of antibiotics against *Burkholderia pseudomallei*. *Int. J. Anti. Agents. 41(4):* 330-336

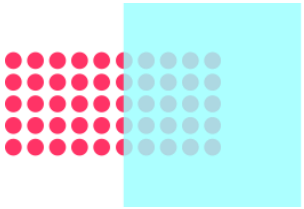
UNIVERSITY OF EXETER

Bottle neck in discovery of lead compounds



Actinomycete collection

HPLC purification of candidates



toxicity screen in cell culture

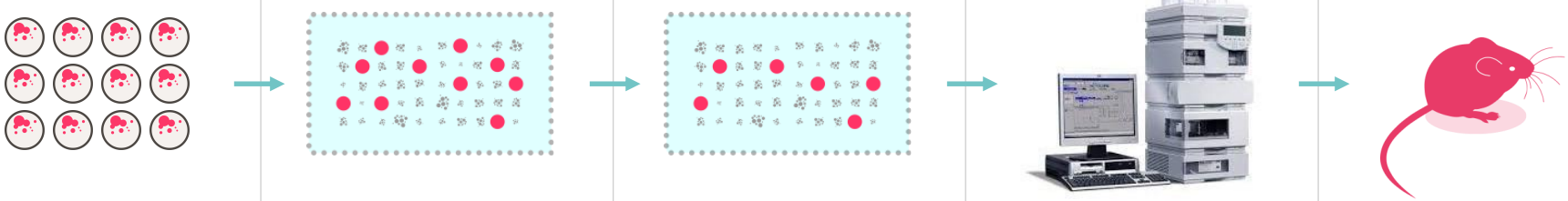
efficacy in mice

UNIVERSITY OF EXETER

Demuris

BioSystems TECHNOLOGY

Rapid lead selection pipeline



Actinomycete collection

Crude extract efficacy screen in Galleria

Crude extract toxicity screen in Galleria

HPLC purification of candidates

Efficacy and toxicity in mice

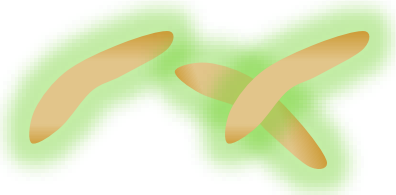
UNIVERSITY OF EXETER

Demuris

BioSystems TECHNOLOGY

The future

Tools to genetically engineer larvae



Technologies to enable high throughput screening

AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements™

EUKARYOTES

Genome Sequence of *Galleria mellonella* (Greater Wax Moth)

Anna Lange,[a] Sina Beier,[b] Daniel H. Huson,[b] Raphael Parusel,[a] Franz Iglauer,[a] Julia-Stefanie Frick[a]

[a]Department for Medical Microbiology and Hygiene, Interfaculty Institute for Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany
[b]Algorithms in Bioinformatics, ZBIT Center for Bioinformatics, University of Tübingen, Tübingen, Germany
[c]Einrichtung für Tierschutz, Tierärztlichen Dienst und Laboratorientkunde, Eberhard Karls Universität Tübingen, Tübingen, Germany

ABSTRACT The larvae of the greater wax moth, *Galleria mellonella*, are pests of active beehives. In infection biology, these larvae are playing a more and more attractive role as an invertebrate host model. Here, we report on the first genome sequence of *Galleria mellonella*.

A ubiquitous pest of beehives, the greater wax moth causes severe damage due to its destructive way of feeding (1). As an invertebrate host model, it is used to study the virulence of different pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (2), *Listeria monocytogenes* (3), and *Candida* spp. (4). So far, molecular biological studies of gene expression in *Galleria mellonella* have been based on a published transcriptome data set (5), and there was no genome sequence available. However, a genome sequence is crucial to enable homology studies between *Galleria mellonella* and human, mouse, and other model hosts. Here, we describe the first draft genome sequences available for *Galleria mellonella* (isolate FT-Tue), based on PacBio technology. Genomic DNA was extracted using the Qiagen Genomic-tip 100/G kit. Sequencing was performed at GATC Biotech AG (Constance, Germany) using PacBio long-read technology. A PacBio standard genomic library was sequenced on 22 single-molecule real-time (SMRT) cells. After subread filtering, this resulted in a total of 20,638,932,410 bases, 2,141,900 reads, an N_{50} read length of 13,454 bp, and a mean length of 9,635 bp. Our assembly of the genome produced 1,937 contigs comprising 578 Mbp, with an N_{50} of 952 kbp. The largest contig was 8.98 Mbp.

This first draft genome sequence will help to promote the use of *G. mellonella* as a replacement organism for vertebrates in biomedical research.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number NTHM00000000. The version described in this paper is version NTHM01000000.

ACKNOWLEDGMENTS

This work was supported by GV-SOLAS (Society of Laboratory Animal Science), the DFG (grants DFG FR 2087/6-1, DFG FR 2087/8-1, CRC685, and SPP1656), DFG Research Training Group 1708, the Bundesministerium für Bildung und Forschung (BMBF), and the German Center for Infection Research (DZIF).

Received 27 September 2017 **Accepted** 10 November 2017 **Published** 11 January 2018

Citation Lange A, Beier S, Huson DH, Parusel R, Iglauer F, Frick J-S. 2018. Genome sequence of *Galleria mellonella* (greater wax moth). Genome Announc 6:e01220-17. https://doi.org/10.1128/genomeA.01220-17.

Address correspondence to Daniel H. Huson, daniel.huson@uni-tuebingen.de, or Julia-Stefanie Frick, julia-stefanie.frick@med.uni-tuebingen.de.

A.L. and S.B. share first authorship.

UNIVERSITY OF EXETER

Summary

Galleria mellonella larvae are susceptible to infection by a range of bacterial and fungal pathogens

---

The model can reflect differences in the virulence of different strains / mutants (in humans)

The model can be used to screen antimicrobial drugs for efficacy

---

Cost effective, ethical, high throughput

[dstl]

Innovate UK

eurostars™

DTRA
Defense Threat Reduction Agency

NC 3Rs
National Centre for the Replacement Refinement & Reduction of Animals in Research

Acknowledgements

UNIVERSITY OF EXETER

- Steve Michell
- Matt Wand
- Rachel Thomas
- Sariqa Wagley

evolva

- Stan Goldman

Mahidol University

- Muthita Vanaporn

BioSystems TECHNOLOGY

- Olivia Champion