SCIENTIFIC PROGRAMME
ABSTRACT BOOK

GmW

Galleria mellonella WORKSHOP

www.gmw2021.it

WEBINAR CONGRESS

JULY 16th, 2021
from 09.00 to 15.30

JULY 17th, 2021
from 09.00 to 12.30

Galleria mellonella Workshop

This two-day workshop will bring together users of, and those interested in using, the model host *Galleria mellonella,* for an unparalleled opportunity to learn from, be inspired by and network with the international research community.
Galleria mellonella larvae can be used as an economical, rapid, high-throughput model to bridge the gap between in vitro studies and mammalian research, thus improving preclinical studies and reducing the number of mammals used in drug testing. *G. mellonella* larvae have been widely used over the past few years as non-mammalian models of microbial infection and for antimicrobial drug screening.
This is particularly relevant as NC3Rs have announced their research highlight for 2019: the use of alternative research models such as *G. mellonella.*

Under the patronage of

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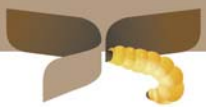
Antibatterica - Antivirale - Antifungina



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Eleftherios Mylonakis
Brown University, Rhode Island, US

Christina Nielsen-LeRoux
INRAE Center of Jouy en Josas, Paris, France

Emerenziana Ottaviano
University of Milan, Italy

Richard Titball
University of Exeter, UK

Andreas Vilicinskas
Justus Liebig University of Giessen, Germany

July 16th, 2021 - Day 1

08.45-09.00 Registration of participants
(access and connection to web platform)

09.00-09.15 Welcome and Introduction to the meeting
S. Centanni (Milan, Italy)

SESSION 1 *GALLERIA* AS AN INFECTION MODEL

Chair: **R. Titball** (Exeter, UK)**, C. Nielsen-LeRoux** (Paris, France)

09.15-09.40 Lecture 1 - Characterization of the processes leading to *Madurella mycetomatis* grain transformation in *Galleria mellonella* larvae
K. Kavanagh (Maynooth, Ireland)

09.40-10.10 Lecture 2 - *Galleria mellonella* as an infection model for the *Mycobacterium tuberculosis* complex
P. Langford (London, UK)

10.10-10.30 Lecture 3 - Omics based study of *Coxiella burnetiid* infection
R. Titball (Exeter, UK)

10.30-10.40 Discussion

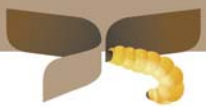
10.40-11.00 **Break**

SESSION 2 ORAL PRESENTATIONS (Oral Communications and Oral Poster Presentations)

Chairs: **E. Borghi** (Milan, Italy), **E. Ottaviano** (Milan, Italy)

11.00-11.05 **OC 1 -** *GALLERIA MELLONELLA* AS A NOVEL CONTAINMENT LEVEL 3 MODEL TO STUDY *MYCOBACTERIUM TUBERCULOSIS* H37RV - *M. Asai, Y. Li, J. Spiropoulos, W. Cooley, D. Everest, B.D. Robertson, P.R. Langford, S.M. Newton*

11.05-11.10 **OPP 1 -** *GALLERIA MELLONELLA* AS AN INFECTION MODEL THAT DEMONSTRATES THE PATHOGENIC POTENTIAL OF *STREPTOCOCCUS ANGINOSUS* GROUP - *J. Budziaszek, M. Pilarczyk-Zurek, I. Sitkiewicz, and J. Koziel*



11.10-11.15 **OC 2 -** TOWARDS BETTER CHARACTERISATION OF THE *GALLERIA* IMMUNE SYSTEM - *J. Campbell, J. Pearce, I. Cañada Luna, J. Wakefield*

11.15-11.20 **OC 3 -** THE LIFE INTRACELLULAR - USING *GALLERIA MELLONELLA* TO INTERROGATE HOST PHAGOCYTE-MICROBE INTERACTIONS - *A.M. Krachler, N. Sirisaengtaksin, C.J. Coates, J. Lim*

11.20.11.25 **OC 4 -** *GALLERIA MELLONELLA* AS VALUABLE INFECTION MODEL TO EVALUATE THE *PISTACIA VERA* L. OLEORESIN AND LEVOFLOXACIN SYNERGISTIC COMBINATIONS AGAINST RESISTANT *HELICOBACTER PYLORI* STRAINS - *S. Di Lodovico, E. Di Campli, P. Di Fermo, S. D'Ercole, A. Nostro, G. Magi, M. Di Giulio, L. Cellini*

11.25-11.30 **OPP 2 -** COMPARATIVE ANALYSIS *IN VIVO* USING THE LARVAE MODEL *GALLERIA MELLONELLA* TO ASSESS THE VIRULENCE PROFILES OF ENVIRONMENTAL AND CLINICAL *VIBRIO PARAHAEMOLYTICUS* - *A. Hughes*

11.30-11.35 **OC 5 -** *GALLERIA MELLONELLA* AS A MODEL HOST TO STUDY EPIGENETIC BASIS OF DISEASES - *K. Mukherjee and U. Dobrindt*

11.35-11.40 **OPP 3 -** *GALLERIA MELLONELLA* AS A MODEL FOR EVALUATING THE VIRULENCE OF *ACHROMOBACTER XYLOSOXIDAN*S CLINICAL STRAINS FROM CYSTIC FIBROSIS PATIENTS - *R. Passarelli Mantovani, A. Sandri, G. Burlacchini, M. Boaretti, P. Melotti, C. Signoretto, M.M. Lleo*

11.40-11.45 **OC 6 -** VIRULENCE QUANTIFICATION OF KPC- AND OXA-48-PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATES IN A *GALLERIA MELLONELLA* MODEL: TOWARDS A NOVEL THERAPEUTIC APPROACH USING LINEAR CATIONIC POLYMERS - *D. Mil-Homens, M. Martins, J. Barbosa, M.J. Sarmento, R. Pires, V. Rodrigues, V.D.B. Bonifácio, S.N. Pinto*

11.45-11.50 **OPP 4 -** *GALLERIA MELLONELLA* AS A MODEL HOST TO STUDY THE VIRULANCE OF *FUSARIUM MUSAE* STRAINS OBTAINED FROM PLANTS AND HUMANS - *V. Tava, E. Vanhoffelen, A. Reséndiz Sharpe, H. Hendrix, R. Lavigne, K. Lagrou, M. Pasquali, G. Vande Velde*

11.50-11.55 **OC 7 -** *GALLERIA MELLONELLA* LARVAE AS AN EFFICIENT *IN VIVO* MODEL TO CHARACTERIZE THE EFFICACY OF ANTIMICROBIAL PEPTIDES AGAINST MULTI-DRUG RESISTANT ENTEROAGGREGATIVE *E. COLI - J. Vergis, S.V.S. Malik, R. Pathak, M. Kumar, N.V. Kurkure, S.B. Barbuddhe, and D.B. Rawool*

11.55-12.00 Discussion

12.00-13.15 **Break**

SESSION 3 DRUG DISCOVERY

Chair: **E. Mylonakis** (Rhode Island, US)**, R. Titball** (Exeter, UK)

13.15-13.45 Lecture 4 - *Galleria mellonella*-derived antibicrobial peptides a new antibiotic lead molecules
A. Vilcinskas (Giessen, Germany)

13.45-14.15 Lecture 5 - *Galleria mellonella* as a novel *in vivo* drug discovery platform using bioluminescent KAPE pathogens
O. Champion (Torquay, Devon UK)

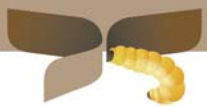
14.15-14.45 Lecture 6 - Anti-MRSA Compounds Identified Using a Whole-Animal *Caenorhabditis elegans/Galleria mellonella* Sequential-Screening Approach
E. Mylonakis (Rhode Island, US)

14.45-14.50 **OC 8 -** PIGGYBAC AND CRISPR/CAS9 MEDIATED GENOME EDITING IN *GALLERIA MELLONELLA - J. Pearce, J. Prior, R. Titball, J. Wakefield*

14.50-15.05 Discussion

15.05-15.30 Conclusions

END OF THE FIRST DAY



SESSION 4 MICROBIOTA AND GASTROINTESTINAL PATHOGENS

Chair: **O. Champion** (Torquay, Devon, UK)**, A. Vilicinskas** (Giessen, Germany)

09.30-09.50 Lecture 7 - *Galleria mellonella* gut microbiota manipulation impacts on larval development
E. Borghi (Milan, Italy)

09.50-10.20 Lecture 8 - *Galleria mellonella* as a model for intestinal infection and histology studies
C. Nielsen-LeRoux (Paris, France)

10.20-10.25 **OPP 5 -** THE POSTBIOTIC ACTIVITY OF *LACTOBACILLUS PARACASEI* 28.4 AGAINST *CANDIDA AURIS* - *L.M.A. Figueiredo-Godoi, R.D. Rossoni, P. Pimentel de Barros, I. do Carmo Mendonça, R. Previate Mendonça, D.H. Siqueira Silva, B. Burgwyn Fuchs, J. Campos Junqueira, E. Mylonakis*

10.25-10.30 **OC 9 -** DEVELOPING *GALLERIA MELLONELLA* AS A MODEL FOR THE INFANT GUT MICROBIOME - *H. Gooch*

10.30-10.40 Discussion

10.40-10.50 **Break**

SESSION 5 *GALLERIA* AS BIOFILM – RELATED INFECTION MODEL

Chair: **K. Kavanagh** (Maynooth, Ireland)**, E. Mylonakis** (Rhode Island, US)

10.50-11.20 Lecture 9 - Foreign body infection model in *Galleria mellonella* larvae implanted with stainless steel K-wires
M. Di Luca (Pisa, Italy)

11.20-11.50 Lecture 10 - Exploring the *Galleria mellonella* model to study antifungal therapies for oral candidiasis
J.C. Junqueira (Sao Paolo, Brazil)

July 17th, 2021 - Day 2

11.50-11.55 **OC 10 -** AN INVERTEBRATE BURN WOUND MODEL THAT RECAPITULATES THE HALLMARKS OF BURN TRAUMA AND INFECTION SEEN IN MAMMALIAN MODELS - *E. Maslova, Y. Shi, F. Sjöberg, H.S. Azevedo, D.W. Wareham and R.R. McCarthy*

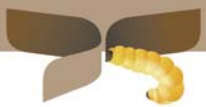
11.55-12.00 **OC 11 -** TOWARDS BIOLUMINESCENCE IMAGING AS AN OBJECTIVE AND DYNAMIC READOUT OF FUNGAL LOAD IN A *GALLERIA MELLONELLA* MODEL OF ASPERGILLOSIS - *E. Vanhoffelen, A. Resendiz-Sharpe, V. Tava, H. Hendrix, R. Lavigne, K. Lagrou, G. Vande Velde*

12.00-12.05 **OC 12 -** METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* USA300 PERSISTER CELLS SHOW CHAPERONE UPREGULATION IN CONTRAST TO PLANKTONIC CELLS AND THE BIOFILM PHENOTYPE - *J. Vlaeminck, B.B. Xavier, Q. Lin, S. De Backer, H. De Greve, S. Kumar-Singh, H. Goossens & S. Malhotra-Kumar*

12.05-12.15 Discussion

12.15-12.30 Conclusions

END OF WORKSHOP



DATES OF CONGRESS

Friday July 16th, 2021, from 09.00 to 18.00
Saturday July 17th, 2021, from 09.00 to 12.30

SCIENTIFIC SECRETARIAT

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ORGANIZING SECRETARIAT

NADIREX INTERNATIONAL srl
CONGRESSI - MEETING - COMUNICAZIONE

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ONLINE REGISTRATION

www.gmw2021.it

The registration includes:
- Participation at webinar congress works
- Attendance certificate
- Post-event educational materials
- Assistance of a technician before and during the webinar Congress

REGISTRATION FEES

€ 100,00* (€ 81,97 + 22% VAT)

* Undergraduate and post graduate students are eligible for the reduced student registration fee.

The delegate and student registration fee includes entrance to all of the workshop sessions, lunch and coffee in the workshop venue. Accommodation and travel is NOT included in the registration fee.

METHODS OF PAYMENT

The registration fee can be paid with:
- Bank transfer headed to: Nadirex International S.r.l.
  c/o: Intesa Sanpaolo - Filiale di Pavia - Viale Cesare Battisti 18
  IBAN: IT66L0306911310100000069654
  BIC: BCITITMM - C/C Number: 100000069654
  Please send a copy of payment by e-mail at info@nadirex.com
  or by fax: +39 0382525736
- Credit Card: Visa - Master Card - American Express

CERTIFICATE OF ATTENDANCE

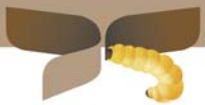
You will receive your certificate of attendance by an e-mail from Organizing Secretariat by the end of the Workshop.

POSTERS

They will be available in digital format at www.gmw2021.it
The Organizing Secretariat will send to all participants ID and PASSWORD for download.

GUIDELINES FOR SPEAKERS AND CHAIRMEN

The Zoom room will be opened only for speakers and chairmen at:
Friday 16th July: 07.30 a.m.
Saturday 17th July: 08.00 a.m.



ABSTRACTS AND POSTERS

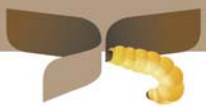
OC1

GALLERIA MELLONELLA AS A NOVEL CONTAINMENT LEVEL 3 MODEL TO STUDY *MYCOBACTERIUM TUBERCULOSIS* H37RV

M. Asai[1], Y. Li[1], J. Spiropoulos[2], W. Cooley[2], D. Everest[2], B.D. Robertson[3], P.R. Langford[1], S.M. Newton[1]

1 *Section of Paediatric Infectious Disease, Department of Infectious Disease, Imperial College London, London, UK*
2 *Department of Pathology, Animal and Plant Health Agency, Addlestone, UK*
3 *MRC Centre for Molecular Bacteriology and Infection, Department of Infectious Disease, Imperial College London, London, UK*

Mammalian infection models have contributed significantly to our understanding of the host- mycobacterial interaction, revealing potential mechanisms and targets for novel antimycobacterial therapeutics. However, the use of conventional mammalian models such as mice, are typically expensive, high maintenance, require specialised animal housing, and are ethically regulated. The insect larvae of *Galleria mellonella* (greater wax moth), has become increasingly popular as an infection model and we previously demonstrated their potential as a mycobacterial infection model using *Mycobacterium bovis* BCG and a double auxotrophic mutant (SAMTB) of *Mycobacterium tuberculosis* (MTB) both compliant for Containment level (CL) 2 conditions. Here, we present a novel CL3 infection model to study the most widely used MTB strain in tuberculosis (TB) research, H37Rv. Our results show a H37Rv dose-dependent survival of G. mellonella larvae and demonstrate growth and persistence of H37Rv over an 8 day infection time-course. In comparison to BCG and SAMTB, H37Rv was more virulent. The use of transmission electron microscopy visualised the rapid interaction between haemocytes and H37Rv bacilli as early as 1 h post-infection. We additionally demonstrate the drug efficacy of clinically recommended antimycobacterial drugs, and via knockout mutants the use of the model for comparative virulence studies. Our findings demonstrate the broad potential of this insect model to study MTB infection under CL3 conditions. We anticipate that the availabilities of mycobacterial models at both CL2 and CL3 conditions will lead to successful adaptation in the broader TB researching community.



OPP1

GALLERIA MELLONELLA AS AN INFECTION MODEL THAT DEMONSTRATES THE PATHOGENIC POTENTIAL OF *STREPTOCOCCUS ANGINOSUS* GROUP

J. Budziaszek[1] , M. Pilarczyk-Zurek[1] , I. Sitkiewicz[2] , and J. Koziel[1]

1 *Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland.*
2 *Department of Drug Biotechnology and Bioinformatics, National Medicines Institute, Chełmska 30/34, 00-725 Warsaw, Poland*

Streptococcus anginosus group (SAG), formerly known as *Streptococcus milleri,* consists of three distinct streptococcal species: *Streptococcus anginosus, Streptococcus constellatus,* and *Streptococcus intermedius.* SAG was considered for many years as commensal bacteria of oral cavity, colon, and genitourinary system. However, a recent observation reported those bacteria as potent pathogens forming brain or liver abscesses. The mechanism of pathogenesis of SAG is still unknown, despite the strong set of clinical data. We established and characterized the infection process of selected SAG isolates in the model of wax worm *Galleria mellonella* to examine their virulence potential. In our studies, we analyzed: (i) the bacterial survival in the larvae hemolymph; (ii) the kinetic of infection process studying survival, activity, and melanization of larvae after bacterial infection. Moreover, we characterized the innate immune response of *G. mellonella* to SAG estimating: hemocytes infiltration and activation of signaling pathways manifested by the expression of gallerimycin and galiomycin. To evaluate specific morphological features of larvae's tissues after infection hematoxylin-eosin and Gram staining were performed. Obtained results indicated that *G. mellonella* could be applied to a fast and effective screening of SAG virulence. Moreover, we found *G. mellonella* as a convenient model for more detailed studies of the corruption of innate immunity by SAG.

Supported by National Science Centre, Poland 2018/29/B/NZ6/00624

OC2

TOWARDS BETTER CHARACTERISATION OF THE *GALLERIA* IMMUNE SYSTEM

J. Campbell*, J. Pearce, I. Cañada Luna, J. Wakefield

** Presenting, University of Exeter*

The use of model organisms is vital if we are to understand immune cell activation and infection responses within the context of the complex and dynamic host environment. *Galleria mellonella* has emerged as a promising partial replacement model for the study of human pathogens, with the distinct advantages of being inexpensive and viable at 37 °C. Generally, studies into infection outcomes using *Galleria* are limited to total immune cell counts, organism melanisation and, ultimately, death – although newer studies involving immune cell transcriptomics are beginning to emerge.

In the Wakefield Lab we have begun to characterise the *Galleria* immune cells – termed hemocytes – further. Using both flow cytometry and FACs sorting we have isolated subpopulations of hemocytes based on internal complexity and phagocytic uptake of fluorescent zymosan particles. We find that these cells express GATA and Runt-domain transcription factors which are orthologous to genes found in the well characterised immune cells of *Drosophila melanogaster,* as well as several phagocytic receptors of interest. Using these genes as readouts via RT-PCR, we hope to be able to investigate changes to both the immune cell landscape and immune cell activation to better understand how the host responds to immune challenges on a molecular level.

Furthermore, our preliminary investigations into gene expression in Galleria hemocytes has identified potential target promoters for the generation of immune cell specific transgenic animals. We have begun this work using PiggyBac mediated DNA integration with the aim to generate lines with fluorescent hemocytes for further characterisation by in vivo microscopy and FACs. We hope that this will increase the use of *Galleria mellonella* in infection studies and diversify the experiments possible with this replacement model.

OC3

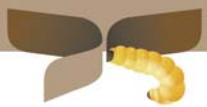
THE LIFE INTRACELLULAR - USING *GALLERIA MELLONELLA* TO INTERROGATE HOST PHAGOCYTE-MICROBE INTERACTIONS

A.M. Krachler[1], N. Sirisaengtaksin[1], C.J. Coates[2], J. Lim[3]

1 University of Texas McGovern Medical School at Houston, USA
2 College of Science, Wales UK
3 University of Stirling, Scotland UK

Experimental measures such as survival, movement, pupation and melanisation extent are commonplace when using insect larvae to discriminate between virulent and non-virulent disease-causing agents, and their ecotypes.
Recently, we have focussed our efforts on host-pathogen interactions at both the organismal and cellular (haemocyte, macrophage) levels, and the extent to which intracellular pathogens like *Cryptococcus neoformans* and *Yersinia pseudotuberculosis* modulate the innate immune responses.
Adhesins facilitate bacterial colonization and invasion of host tissues and are thus considered virulence factors, but their impact on immune-mediated damage as a driver of pathogenesis is often unclear. *Yersinia pseudotuberculosis* causes zoonotic infections, and phagocyte invasion is essential for bacterial persistence.
Y. pseudotuberculosis encodes for a multivalent adhesion molecule (MAM), a mammalian cell entry (MCE) family protein and adhesin. MAMs are wide-spread in Gram-negative bacteria, and highly conserved amongst *Yersinia* spp. MAM adhesins facilitate colonization of epithelial tissues by enteric bacteria, but their role in bacterial interactions with the host innate immune system and contribution to *Y. pseudotuberculosis* pathogenicity remains unclear. Here, we investigated how *Y. pseudotuberculosis* MAM impacts bacteria – innate immune interactions and pathogenicity using *Galleria mellonella* larvae as a host.
We show that *Y. pseudotuberculosis* MAM is required for efficient bacterial association with and invasion of phagocytes. We demonstrate that *Y. pseudotuberculosis* interactions with insect- and mammalian phagocytes are determined by analogous bacterial- and host factors.
Loss of MAM decreased bacteria-driven pathogenesis in *G. mello-*

nella. Diminished phagocyte invasion led to increased bacterial clearance, and a lower bacterial burden.
However, invasion-deficient *Y. pseudotuberculosis* hyper-activated humoral immune responses, most notably melanin production, which, despite clearing the pathogen, caused increased phagocyte death and higher host mortality. Our findings provide experimental evidence for the applicability of the damage-response framework to non-vertebrate hosts.



OC4

GALLERIA MELLONELLA AS VALUABLE INFECTION MODEL TO EVALUATE THE *PISTACIA VERA* L. OLEORESIN AND LEVOFLOXACIN SYNERGISTIC COMBINATIONS AGAINST RESISTANT *HELICOBACTER PYLORI* STRAINS

S. Di Lodovico, E. Di Campli, P. Di Fermo, S. D'Ercole*, A. Nostro, G. Magi***, M. Di Giulio, L. Cellini**

Departments of Pharmacy and of
**Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" Chieti-Pescara, Italy*
***Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy*
****Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona, Italy*

Aim: *Galleria mellonella* is a recognized infection model to study the pathogenesis of bacterial or fungal infections and, in particular, it is a validate model to study the *Helicobacter pylori* virulence.
Helicobacter pylori is a gastroduodenal pathogen, difficult to treat, characterized by an increasing multidrug resistance also correlated to its biofilm-forming ability. The increase of antimicrobial resistance and the failure of therapeutic regimens, strongly underline the need to find novel strategies to improve the management of the microbial disease. Many studies demonstrate that natural compounds can act as effective enhancers of therapeutic schemes, enhancing the eradication rate.
On the bases of these considerations, the aim of this work was to evaluate, *in vitro* and *in vivo,* the *Pistacia vera* L. oleoresin (ORS) capability to synergize with levofloxacin (LVX) against resistant *H. pylori* strains.
Methods used: The *in vitro* antimicrobial and antivirulence activities of ORS, LVX and their synergistic combinations were determined by MIC/MBC, checkeboard test and biomass quantification.
The ORS toxicity was evaluated with *G. mellonella* model in terms of larvae survival percentages, treated with several doses of ORS, every day, until 5 days.
For the *in vivo* infection assay, the survival percentages of *G. mellonella,* infected with lethal dose of *H. pylori* and treated then with ORS alone and combined with LVX, were checked every day, until 5 days. Larvae were considered dead when were unresponsive to touch. The effect of

ORS and LVX against *H. pylori* infection was also performed by spreading diluted hemolymphs extracted from treated larvae.
Results and conclusions: ORS showed a moderate antimicrobial action and it was able to synergize with LVX, restoring its effectiveness in LVX resistant *H. pylori* strains. In particular, ORS and LVX MICs ranged from 780 to 3120 mg/l and from 0.12 to 1.00 mg/l, respectively. MBCs were similar to MICs. ORS was able to synergize with LVX restoring its effectiveness in all resistant detected strains with FIC Index from 0.18 to 0.50. Moreover, ORS, LVX and their synergistic combinations displayed significant microbial biofilm reductions up to 60.45% at 1/2 MIC.
ORS can be considered as not toxic compound with *G. mellonella* survival percentage from 60% to 80% at maximum dose (1000mg/kg). The treatment with LVX rescue larvae injected with *H. pylori* with a survival rate between 90% and 100%. After treatment with ORS, the larvae survival percentage ranged from 62% to 75% after 5 days. The best synergistic combination of ORS plus LVX shows a protective effect against *H. pylori* infection with larvae survival rate of 63% and 90% after 5 days (fig. 1). This data was also confirmed by the low bacteria CFU recovered from *G. mellonella* after treatment at different time point (fig. 2).

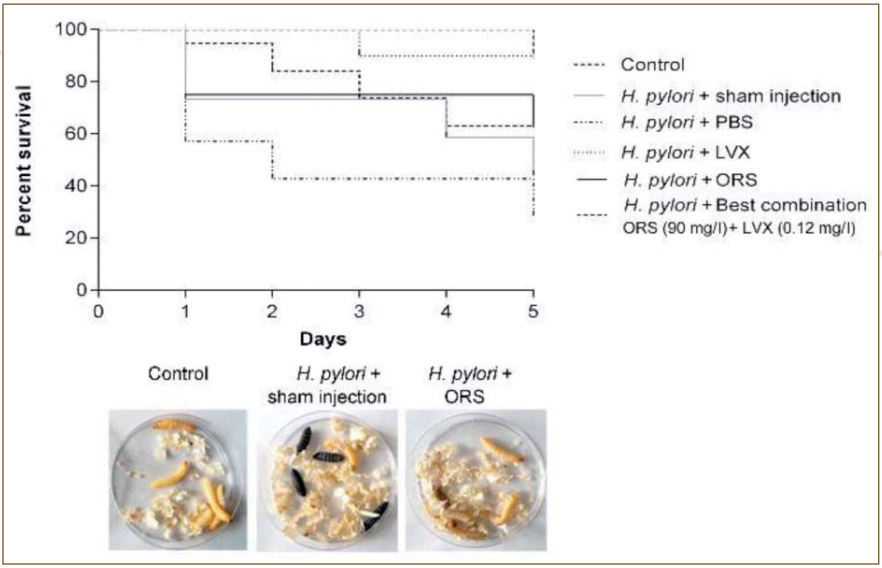


Fig. 1 - Kaplan-Meier survival curves of *G. mellonella* larvae after infection with 1.8 x 106 CFUs.

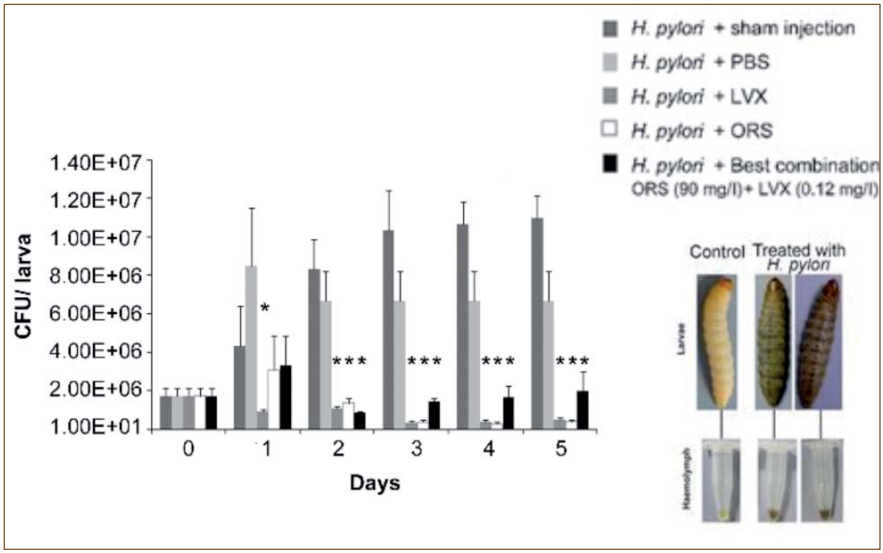


Fig. 2 - Recovery of *H. pylori* in *G. mellonella* larvae after injection with 1.8 x 106 CFUs.

Pistacia vera L. ORS, both alone and combined with LVX, showed a protective effect against *H. pylori* infection over time confirming its effect also in *in vivo* model.

The combined administration of ORS and LVX results in a significant reduction of the antibiotic that is efficacious at concentration lower than its breakpoint value. Overall, ORS can be considered a promising potentiator for restoring, *in vitro* and *in vivo,* the effectiveness of LVX through a synergistic action, tackling the H. pylori antibiotic resistance phenomenon.

These results are obtained by using standardized *in vitro* methodologies and are validated *in vivo* by *G. mellonella* larvae that represent a simple, reliable and reproducible model.

Keywords: *Galleria mellonella, Helicobacter pylori, Pistacia vera* L. oleoresin, Microbial resistance, Synergistic combinations.

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OPP5

THE POSTBIOTIC ACTIVITY OF *LACTOBACILLUS PARACASEI* 28.4 AGAINST *CANDIDA AURIS*

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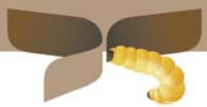
Candida auris has emerged as a medically important pathogen with considerable resistance to antifungal agents. The ability to produce biofilms is an important pathogenicity feature of this species that aids escape of host immune responses and antimicrobial agents. The objective of this study was to verify antifungal action using *in vitro* and *in vivo* models of the *Lactobacillus paracasei* 28.4 probiotic cells and postbiotic activity of crude extract (LPCE) and fraction 1 (LPF1), derived from *L. paracasei* 28.4 supernatant. Both live cells and cells free supernatant of *L. paracasei* 28.4 inhibited *C. auris* suggesting probiotic and postbiotic effects. The minimum inhibitory concentration (MIC) for LPCE was 15 mg/mL and ranges from 3.75 to 7.5 mg/mL for LPF1. Killing kinetics determined that after 24 h treatment with LPCE or LPF1 there was a complete reduction of viable *C. auris* cells compared to fluconazole, which decreased the initial inoculum by 1-logCFU during the same time period. LPCE and LPF1 significantly reduced the biomass ($p=0.0001$) and the metabolic activity ($p=0.0001$) of *C. auris* biofilm. There was also a total reduction (~108CFU/mL) in viability of persister *C. auris* cells after treatment with postbiotic elements ($p<0.0001$). In an *in vivo* study, injection of LPCE andLPF1 into *Galleria mellonella* larvae infected with *C. auris* prolonged survival of these insects compared to a control group ($p<0.05$) and elicited immune responses by increasing the number of circulating hemocytes and gene expression of an-

timicrobial peptide galiomicin. We concluded that the *L. paracasei* 28.4 cells and postbiotic elements (LPCE and LPF1) have antifungal activity against planktonic cells, biofilms, and persister cells of *C. auris.* Postbiotic supplementation derived from *L. paracasei* 28.4 protected *G. mellonella* infected with *C. auris* and enhanced its immune status indicating a dual function in modulating a host immune response.



OC9

DEVELOPING *GALLERIA MELLONELLA* AS A MODEL FOR THE INFANT GUT MICROBIOME

H. Gooch

John Innes Center

The human microbiome is rapidly becoming recognised as a central player in human health, with both a metabolic and immunological function. The make-up of the gut microbiome, especially in infants, can be a major factor predisposing individuals to diseases such as IBD, diabetes, and necrotising enterocolitis. Recent improvements in sequencing technologies and computational analysis of sequencing data have allowed scientists to see in detail how changes in the microbiome accompany different lifestyles and health conditions. However, the causality and mechanisms of these associations are still not fully understood. There is also a need to develop and test potential therapeutics targeting the microbiome.

Mice are the most commonly used in vivo model for the microbiome but pose issues in terms of cost, time and ethics. My project aims to develop *Galleria* as an alternative model for the human infant gut microbiome. Previous experiments carried out in the Maxwell lab show that the *Galleria* microbiome can be cleared of native bacteria and colonised with infant gut bacteria. The current work aims to repeat and refine these experiments. So far, we have successfully colonised the *Galleria* gut with two pathobionts from the infant gut through feeding faecal slurry: *Proteus mirabilis* and *Enterococcus faecium*. We are now attempting to develop *Galleria* as a model for *Enterococcus* commensal colonisation of the gut in order to investigate *Enterococcus* virulence and the evolution of antibiotic resistance.

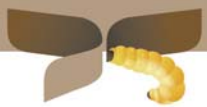
OPP2

COMPARATIVE ANALYSIS *IN VIVO* USING THE LARVAE MODEL *GALLERIA MELLONELLA* TO ASSESS THE VIRULENCE PROFILES OF ENVIRONMENTAL AND CLINICAL *VIBRIO PARAHAEMOLYTICUS*

A. Hughes

CEFAS

Background. Vibrios are a group of Gram-negative, rod-shaped halophilic bacteria that are found naturally in marine, estuarine and freshwater environments. They grow preferentially in warm (>15°C), low salinity (<25 parts per thousand (ppt) NaCl) waters. Vibrios have the potential to provide a useful tool to monitor climate change, due to their fast replication rate, shown as quickly as 10 minutes in ideal conditions (1), and preference for warmer waters. Increasing sea surface temperatures (SST) provide an ideal platform for the increase in abundance of many *Vibrio* species, this phenomenon is being increasingly observed in latitudes as far north as Finland (2). Over 100 species of *Vibrio* have been described to date, of which around 12 can cause disease in humans, four species of interest include *Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholerae* and *Vibrio alginolyticus.*
V. parahaemolyticus is the leading cause of seafood-associated bacterial gastroenteritis and infections are common worldwide (3,4). Transmission occurs via the ingestion of raw, undercooked or re-contaminated seafood. Symptoms associated with *V. parahaemolyticus* infection include diarrhoea, abdominal cramps, nausea, headache, fever and chills, in healthy individuals, symptoms are usually self-limiting and resolve with 72 hours. Not all strains of *V. parahaemolyticus* are pathogenic to humans, vibrios are natural constituents of fresh, marine and estuarine environments and pathogenic strains can often make up a small proportion of the overall microbial community. Strains of *V. parahaemolyticus* that are pathogenic have two major toxigenic virulence factors, thermostable direct haemolysin (TDH) and thermostable direct-related haemolysin (TRH). Another significant virulence factor associated with this pathogen are two type 3 secretion systems (T3SS1/2), these secretion systems are needle-like apparatus that deliver effector proteins, such as haemolysins and toxins, through the host membrane and into the host cell cytoplasm resulting in cytotoxicity and



enterotoxicity (5–7). Environmental isolates are often non-pathogenic due to their lack of the major virulence factors (6).
Typical methods employed for studying the virulence of *V. parahaemolyticus in vivo* involve vertebrate models such as murine, infant rabbit and rabbit ileal-loop, these models are expensive, require extensive training and have many complex ethical issues surrounding their use (7,8). The Galleria mellonella insect model provides an excellent alternative to the traditional vertebrate models traditionally employed for this type of experimentation., it requires minimal training, it does not require ethical approval, and is much cheaper and easier to maintain. In addition, G. mellonella larvae have similarities in their innate immune response to that seen in vertebrates and are able to tolerate higher temperatures, 37°C – mirroring those observed in human infections, in comparison to other invertebrate models, this advantage allows for temperature dependent genes to be observed.
In this study, we used the *G. mellonella* larvae model to assess and compare the virulence of clinical and environmentally important *V. parahaemolyticus* strains that represent key serotypes of this pathogen circulating globally. Isolates were chosen based on their genotype, ascertained via whole genome sequencing (WGS), in order to visualise and link the genotype of the organism to the phenotypic manifestations of infection. In the wider scheme, this experiment aims to create a baseline of the species and virulence profiles of strains that are circulating locally to the UK and globally to monitor the progression and spread of pathogenic strains in relation to climate warming.
Methods. *V. parahaemolyticus* strains for testing were initially plated on Marine Agar and incubated at 30°C overnight, the following day an overnight culture of the strain was prepared using Marine Broth and incubated in a rotary incubator at 37°C. Prior to inoculation, the bacteria is prepared by washing twice with PBS, then serially diluted to working concentrations. Bacterial plate counts were performed to confirm the dose administered to the larvae.
The larvae were inoculated via microinjection into the right foremost proleg, 30 larvae were tested per strain and 100 colony forming units (CFU) of bacteria were inoculated into each larva. The survival of the larvae was recorded over 48 hours. Healthy larvae are cream coloured and motile, infection in the larvae can be indicated via a reduction in motility and movement, melanisation – turning a mottled grey to black colour, and faecal staining (7). Death is determined by the lack of movement or response when the larva is stimulated with a pipette tip.
Results and conclusions. We have successfully established a testing approach using *V. parahaemolyticus* in *G. mellonella* and have generated

LD_{50} data for a number of clinically important as well as environmental strains. Experimentation is still ongoing, however to date there has been an observed differential killing rate between strains of varying virulence, previously ascertained from clinical studies. In general, toxigenic strains *(tdh/trh+)* demonstrated enhanced virulence, consistent with previous published studies (7). We have successfully achieved co-infections in *G. mellonella*, which will open up the possibility of assessing the role of toxigenic and non-toxigenic strains in the larvae infection model, which to our knowledge has not been attempted before. We noted some differences in highly pathogenic strains inoculated into *G. mellonella,* which is suggestive that repeated passage and sub- culturing in the laboratory may reduce the virulence capabilities of certain strains. This data is invaluable in generating a baseline dataset for comparative virulence purposes. We are currently analysing the virulence data being generated alongside a whole-genome sequence analysis of key pathogenicity genes to provide a means of quantifying the potential contribution of certain virulence markers (e.g. *tdh/trh+,* T6SS). Vibrios are of an important group of emerging foodborne pathogens, of global importance. Currently, there are no global monitoring systems dedicated to Vibrio infection and many infections with *Vibrio* species are transient and do not require medical attention, thus the true burden of infection caused by these bacteria is unknown and is likely to be much higher than currently estimated. Our work represents a key step-change in building up a greater understanding of these pathogens and their virulence capabilities. The establishment of a tractable in vivo testing methodology has many exciting advantages, in that is rapid, easy to use, and circumvents many ethical considerations.

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LECTURE 1

PROTEOMIC ANALYSIS OF THE PROCESSES LEADING TO *MADURELLA MYCETOMATIS* GRAIN FORMATION IN *GALLERIA MELLONELLA* LARVAE

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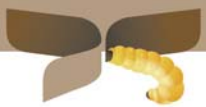
Mycetoma is a devastating, chronic granulomatous infection primarily associated with the fungal pathogen *Madurella mycetomatis*. The infections is endemic in tropical/subtropical regions but the highest incidence is in Africa. Infection results in swelling of the feet/ legs or hands and result in severely reduced mobility and inability to work. Infection is characterised by the formation of fungal grains inside the infected tissue which commonly result in severe deformity and disability. Currently the biochemical processes and interactions between host and pathogen which result in grain formation are poorly characterised. In addition, the infection process in mammals takes months to fully develop making it difficult to study. In order to unravel these processes *Galleria mellonella* larvae were infected with *M. mycetomatis* hyphae, and grain formation, survival, fungal burden and proteomic responses of larvae were monitored for 10 days.

At 24 h post infection proteins indicative of muscle invasion and humoral immune response activation were enriched in infected larval hemolymph. By 72 h immune related hdd11 was increased 337 fold, heat shock proteins 90 was increased 40 fold and glutathione- S-transferase was increased 25 fold. By 7 days post infection proteins which were associated with grain formation (hdd11 [533 fold], hemocentin [54 fold]) and a range of antimicrobial peptides were enriched. During the 7 day period a variety of proteins were decreased in infected hemolymph (e.g. hexamerin, apolipophorin and cationic peptide CP8). This data also identified 75 M. mycetomatis proteins released into hemolymph during infection. Proteins were also extracted from *M. mycetomatis* grains taken from larvae infected for 24, 72 and 7 days. These proteins give an insight into the interactions between the larval immune response and *M. mycetomatis* at



the cellular levels during infection. These results identify similarities between the infection processes of *M. mycetomatis* in *G. mellonella* larvae and in humans and identify novel proteins from M. mycetomatis which may play a crucial role in grain development.
The results of this work indicate that *G. mellonella* larvae are a convenient and useful way to study *M. mycetomatis* grain formation *in vivo* and offer insights into how this process develops in humans.

OC10

AN INVERTEBRATE BURN WOUND MODEL THAT RECAPITULATES THE HALLMARKS OF BURN TRAUMA AND INFECTION SEEN IN MAMMALIAN MODELS

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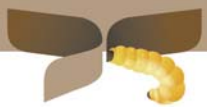
Currently bacterial burn wound infection is the leading reason for mortality among burn patients, especially in burn intensive care units. Burn wound infections also impact the process of recovery, due to longer and more complicated treatment and autograft failures that it induces. A number of animal burn wound models has already been established, e.g. murine model - the most well-known among them. They provided multiple insights into pathogenicity of a wide range of clinically relevant burn pathogens and their interactions with the host. Nevertheless, murine and other animal burn wound models are under firm ethical restrictions, due to the severity and morbidity of the burn injury and infections. Inevitably, a high level of training is required to perform the procedure and to obtain reliable data. This study describes a *Galleria mellonella* burn wound and consequent burn infection model. It demonstrates that this protocol yields the results that follow the hallmarks of burn injury and burn wound infection observed in other animal models and in humans. It also shows its ability to be used to distinguish between low and high pathogenicity strains of Staphylococcus aureus and *Pseudomonas aeruginosa,* in addition to *Acinetobacter baumannii* – three among the most common multidrug-resistant burn wound colonizers. Furthermore, this invertebrate model presents a lesser challenge for ethical approval in comparison to already existing burn wound models and requires less advanced training to use. In addition to that, it allows for high throughput screening of mutant libraries and anti-infective agents.



OC5

GALLERIA MELLONELLA AS A MODEL HOST TO STUDY EPIGENETIC BASIS OF DISEASES

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Institute of Hygiene, University Hospital Muenster, Muenster, Germany

Innate-immunity-related genes in humans are activated during urinary tract infections (UTIs) caused by pathogenic strains of *Escherichia coli* but are suppressed by many commensals. Epigenetic mechanisms play a pivotal role in the regulation of host gene expression in response to environmental stimuli independent of changes in the DNA sequence. Here we present *Galleria mellonella* as a surrogate model host to elucidate the role of epigenetic mechanisms such as DNA methylation, histone acetylation, and microRNA expression in regulating the differential host response to uropathogenic and commensal-like *E. coli* strains. We infected *G. mellonella* larvae with uropathogenic *E. coli* (UPEC) strain CFT073 that causes symptomatic UTIs in humans or the commensal-like attenuated strain 83972 that causes asymptomatic bacteriuria (ABU). Our research shows that infection with the UPEC strain was more lethal to larvae than infection with the attenuated ABU strain due to the recognition of each strain by different Toll-like receptors, ultimately leading to differential DNA methylation, histone acetylation, and miRNA expression in the host. We correlated epigenetic changes with the induction of innate-immunity-related genes. Transcriptomic analysis of infected *G. mellonella* larvae infected with *E. coli* strains CFT073 and 83972 revealed strain-specific variations in the class and the expression levels of genes encoding antimicrobial peptides, cytokines, and enzymes controlling major epigenetic mechanisms. Our results provide evidence for the epigenetic basis of differential regulation of innate immune response in *G. mellonella* larvae when infected uropathogenic or commensal-like *E. coli* strains. Our findings from the surrogate host model serve as a starting point for the characterization of basic regulatory mechanisms involved in the development of symptomatic urinary tract infections in humans.

OPP3

GALLERIA MELLONELLA AS A MODEL FOR EVALUATING THE VIRULENCE OF *ACHROMOBACTER XYLOSOXIDANS* CLINICAL STRAINS FROM CYSTIC FIBROSIS PATIENTS

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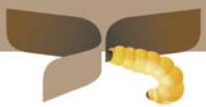
Aims: *Achromobacter xylosoxidans* (ACX) is an emerging pathogen in cystic fibrosis (CF). Although the rate of diagnosis of colonization/infection of the airways with ACX has increased in CF patients, its clinical significance is still unclear. Chronic infection by ACX has been associated with lung inflammation, increased frequency of exacerbations and decline of the respiratory function and is usually complicated by multidrug resistance. Recent studies also indicated the importance of upper airways as possible reservoirs of opportunistic pathogens such as ACX.
In numerous studies, *Galleria mellonella* has been exploited as an alternative host model for investigating virulence factors of different pathogenic bacteria. Because of the relevance of bacterial lung infections in CF patients, the aims of our study was to: i) provide evidence that *G. mellonella* constitutes a useful and convenient model for analysis of the pathogenicity of *A. xylosoxidans* clinical strains, ii) compare the virulence of strains isolated at the same time in two different airway sites in a number of CF patients (nasal lavage and sputum).
Methods: To set up a *G. mellonella* model, we challenged larvae with six different ACX clinical isolates whose virulence was previously confirmed. To identify the optimal growth conditions, bacteria were grown in TSB or BHI. To select the optimal bacterial concentration, ten larvae were inoculated with 4 different doses of bacterial cells (~$2x10^4$-10^7 CFU/larvae) through the last pro-leg into the haemocoel using a 0.3 ml syringe and incubated at 37°C for 72h. Sterile saline and *P. aeruginosa* PAO1 strain were used as negative and positive control, respectively. After identifying the most appropriate conditions, we screened the virulence of 54 ACX clinical strains (43 from sputum and 11 from nasal la-



vage) longitudinally isolated from 14 CF patients (Cystic Fibrosis Center of Verona).

Results and conclusions: The best conditions to distinguish between virulent and non-virulent ACX strains in *G. mellonella* model were established as growth in BHI and infection dose of ~$2x10^5$ CFU/larve. The level of virulence of each strain was defined according to the percentage of larvae death as follows: 27 strains showed no virulence (≤ 20% death), 2 were lowly virulent (30-50% death), 8 expressed moderate virulence (60-80% death), and 17 were highly virulent (≥ 90% death). The strains longitudinally isolated from 9 patients showed to maintain the same level of virulence over time and between the different samples analyzed. In the other 5 patients we observed changes in virulence over time and, in 2 of them, also between isolates from sputum and nasal lavage collected at the same time.

In conclusion, *G. mellonella* larvae proved to be a good infection model to characterize the virulence of ACX strains isolated from the airways of CF patients. The model was useful to identify patients colonized with strains showing a different behaviour between upper and lower airways, that could influence the course of the infection and the outcome of the therapy applied.

OC8

PIGGYBAC AND CRISPR/CAS9 MEDIATED GENOME EDITING IN *GALLERIA MELLONELLA*

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Due to shared aspects of their immunology and physiology, *Galleria mellonella* is a viable alternative to mammalian models in infectious disease research. The larvae of this globally distributed moth are widely available from commercial sources, easy to manipulate and, crucially, can be incubated at a range of temperatures up to 37°C. These advantages allow *Galleria* to reduce the numbers of mammalian models used in microbial infection research with the added potential to replace them in early pharmacological toxicity screening.

However, the lack of genetic tools and transgenic strains for this organism has been a limiting factor to further increasing its use. Here, we demonstrate successful integration of a fluorescent reporter cassette using PiggyBac transposase mediated transgenesis to create a strain expressing both EGFP and DsRed markers. This strain was then used to study the efficacy of CRISPR/Cas9 in *Galleria*, where we were able to knock out EGFP gene function via introduction of in-del mutations in the coding sequence. The changes made by these techniques are heritable and appear stable over multiple generations.

We have already developed lines expressing fluorescent fusion proteins and aim to use utilise these techniques to investigate *Galleria* immunity. Future strains will not only allow the in depth study of *Galleria's* host-pathogen interactions, but also open up its potential use in high throughput screening in drug discovery and cell biology.

OC6

VIRULENCE QUANTIFICATION OF KPC- AND OXA-48-PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATES IN A *GALLERIA MELLONELLA* MODEL: TOWARDS A NOVEL THERAPEUTIC APPROACH USING LINEAR CATIONIC POLYMERS

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Background: *Klebsiella pneumoniae,* one of the most common pathogens found in hospital-acquired infections, is often resistant to multiple antibiotics. In fact, multidrug-resistant(MDR) *K. pneumoniae* producing KPC or OXA-48-like carbapenemases are recognized as a serious global health threat. In this sence, in this study we evaluated the virulence of *K. pneumoniae* aiming potential antimicrobial therapeutics.
Materials/methods: KPC and OXA-48 strains were obtained from patients treated in medical intensive care units in Lisbon, Portugal. The virulence potential of the *Klebsiella pneumonia* clinical isolates was tested in *Galleria mellonella* infection models (Figure 1). For that, *G. mellonella* were inoculated using KPC(+) and OXA-48(+) isolates from the patients. Also, we report for the first time the use of a cationic linear synthetic polymer (L-OEI) for the treatment of KPC- and OXA-48-producing *K. pneumoniae* isolates. The antimicrobial activitiy of L-OEI was evaluated by the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against all isolates. The L-OEI killing mechanism was also investigated.
Results: In the *G. mellonella* model at 48-72 hours, the KPC(+) *K. pneumoniae* isolates were more virulent than the OXA-48(+) *K. pneumoniae* isolates. Virulence was attenuated when low bacterial inoculum (one magnitude lower) were injected in *G. mellonella*. In addition, we also report the use of an antimicrobial polymer (L-OEI) as a promising alternative antimicrobial agent to fight infectious diseases caused by MDR bacteria. L-OEI has a broad-spectrum antibacterial activity and

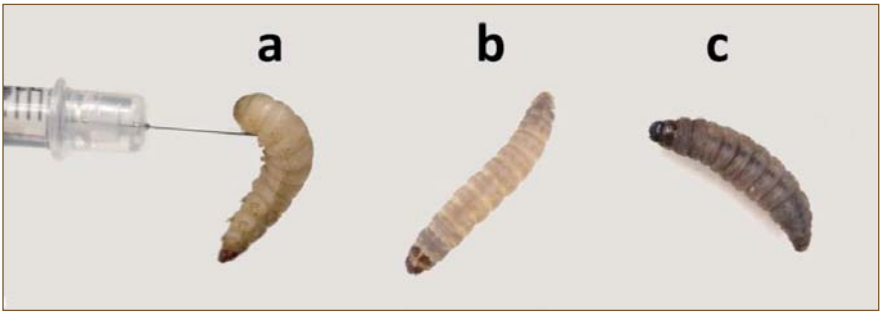
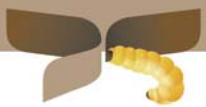


Fig 1: *In vivo* assays using the *Galleria mellonella* larvae model. Inoculation by injection of different bacteria innoculum (a), healthy larvae (b), and death larvae (c) as a result of *K. pneumoniae* infection.

exerts a fast bactericidal activity, by depolarizing the cytoplasmic membrane, against both Gram-negative (including *K. pneumoniae* isolates) and Gram-positive bacteria. Importantly, the polymer does not show toxicity both in vitro (mammalian cell lines) and in vivo (larvae model) under the therapeutic window,

Conclusions: Given its almost negligible toxicity, L-OEI polymer therapeutics may constitute a promising approach for the treatment of MDR *K. pneumoniae* infections.



OPP4

GALLERIA MELLONELLA AS A MODEL HOST TO STUDY THE VIRULANCE OF *FUSARIUM MUSAE* STRAINS OBTAINED FROM PLANTS AND HUMANS

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Fusarium musae is a pathogenic species belonging to the *Fusarium fujikuroi* species complex. Described for the first time only in 2011, *F. musae* is the causative agent of the crown rot on banana, a devastating post-harvest disease, but it also causes keratitis and skin infections as well as systemic infections in immunocompromised patients in few cases. This makes it an ideal species for the comparative analysis of fungal virulence on plant and animal hosts. It's not clear yet how humans acquire the infection, and the absence of experimentally verified proof of the transmission from one host to the other increases the need to build new models to better understand the interaction between the fungus and its hosts.

With this work we aim at creating an in vivo model to further study the mechanisms involved in infection of *Fusarium musae* and to use it as a potential application towards screening system for therapy options. Given strong structural and functional similarities between insect immune system and innate immune response of mammals, insect species such as *Galleria mellonella* have been employed more and more to study microbe-host interactions. Likewise, in this work *Galleria mellonella* is used as a new alternative pathophysiological model to verify infection in a human proxy since it represents an ideal intermediate step between *in vitro* findings and *in vivo* studies in mice for the investigation of human pathogens.

Here we work with 20 different *F. musae* strains collected worldwide from both infected patients and bananas. *G. mellonella* larvae (n=10/group) are injected with 10 µL of five different concentrations of

spores (from 10^2 to 10^6 spores/ml) in order to assess the level of susceptibility of this novel species and compare it with human pathogens already studied (such as *Cryptococcus* and *Aspergillus spp*). They are incubated at different temperatures: 37°C to mimic high body temperature of the host, 30°C that is more representative for skin and corneal infection and 24°C to observe if the temperature can affect fungal virulence. Survival and health score are measured daily for 7 days post infection and colony forming unit (CFU) counts of larval fungal load will be obtained at time of death. Also, the minimum concentration of spores needed to cause symptoms will be established.

The project explores the possibility of considering *G. mellonella* as a successful *in vivo* model for investigation of the initial steps of *Fusarium musae* infection. In this first work we will observe that *Fusarium musae* is actually capable of infecting *G. mellonella* that in this way can be considered as a useful non-vertebrate infection model for studying infection mechanisms of *F. musae* on animal hosts. In addition the virulence of the different strains will be assessed.

Our future work will focus on implementing our findings with the use of bioluminescence as additional readout of the investigation of the mechanisms of action of this pathogen in *Galleria mellonella* firstly but then also in mice. At the end we aim to use *Galleria mellonella* also as a potential application towards screening of therapeutic option since only few treatment options are currently available for *Fusarium* infections.

OC11

TOWARDS BIOLUMINESCENCE IMAGING AS AN OBJECTIVE AND DYNAMIC READOUT OF FUNGAL LOAD IN A *GALLERIA MELLONELLA* MODEL OF ASPERGILLOSIS

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Introduction. *Aspergillus fumigatus* (AF) is an environmental mold that can cause life-threatening respiratory infection in immunocompromised patients. Current antifungal therapies have plateaued in effectiveness, and off-target toxicity and increasing prevalence of azole-resistant AF strains pose a great challenge to clinical success. Therefore, new antifungal treatments are needed[1]. *Galleria mellonella* larvae have been used as an invertebrate model for antifungal screenings to bridge the gap between in vitro findings and in vivo studies in mice. While the translatability of antifungal efficacy and toxicity from *G. mellonella* towards mice is promising, readouts of the *Galleria* model are mostly binary, subjective or invasive[2]. To further optimize antifungal testing in *G. mellonella*, an objective and longitudinal evaluation of fungal load is required. Therefore, we aim to quantify fungal load over time using bioluminescence imaging (BLI) in a *G. mellonella* model of aspergillosis, allowing reliable *in vivo* screening of novel antifungals against azole-resistant and -susceptible AF.

Methods. A genetically modified azole-resistant TR34/L98H AF strain expressing a red-shifted firefly luciferase was used to inoculate healthy sixth instar *G. mellonella* larvae (n=10/group). Larvae were injected with 10 µL of 1 x 10^5 spores AF or NaCl into their hemocoel through the last left proleg, and individually incubated at 37°C. Survival, health index score3 and BLI were obtained daily for 5 days post infection. D-luciferin (in PBS, 0.05 – 0.5 – 5 mg/g) was injected before every BLI acquisition (IVIS Spectrum, Perkin- Elmer) consisting of 30 consecutive images

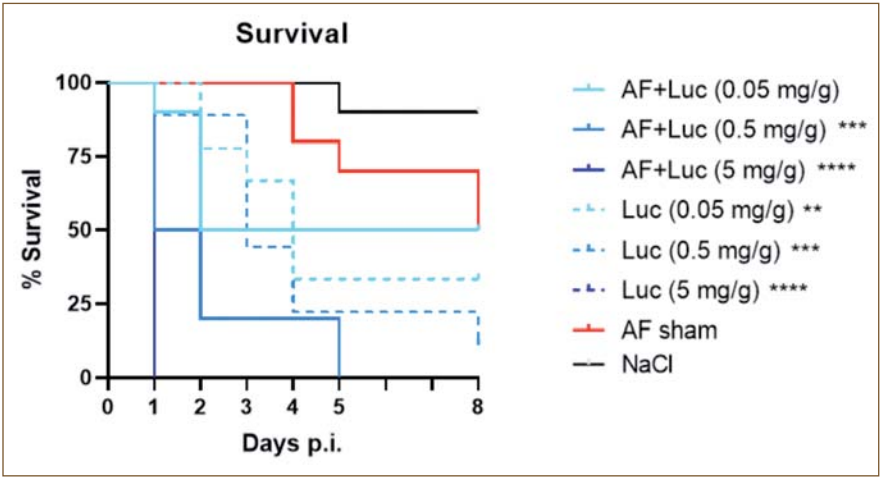

Figure 1. The effect of *Aspergillus fumigatus* infection and D-luciferin on the survival of *G. mellonella* larvae over time. Luciferin 0.05 mg/g, 0.5 mg/g or 5 mg/g was injected to groups of AF infected (1 x 105 spores) and non-infected larvae (n=10/group). Significances are compared to their corresponding controls (Logrank Mantel-Cox test). P.i.: post infection.

with 30 sec exposure. Peak total photon flux (p/s) per larva was determined using Living Image Software version 4.7.3. On the experimental endpoint at day 8, all surviving larvae were sacrificed after BLI acquisition. Colony forming unit (CFU) counts of larval fungal load were obtained at time of death or experimental endpoint, by plating serially diluted larvae homogenates on Sabouraud agar and incubating at 37°C for 48h.

Results & Discussion. First, the toxicity of D-luciferin on *G. mellonella* larvae was assessed by daily injection of AF infected and non-infected larvae with D-luciferin and comparing them to their sham injected controls. In non- infected larvae, D-luciferin caused toxicity in all tested concentrations, visible as decreased survival (Figure 1) and health index score (not shown). However, in AF infected larvae the 0.05 mg/g D-luciferin concentration caused a non-significant decrease in survival (Figure 1) and was therefore provisionally selected to perform BLI on infected larvae. With BLI, we successfully visualized the fungal load of AF in the *G. mellonella* larvae, showing a steady increase in fungal load reaching a maximum at day 4 post infection (Figure 2A, B). Moreover, BLI peak flux and CFU counts were significantly correlated (r=0.75) at time of larval death, validating our BLI readout. This shows the feasibi-

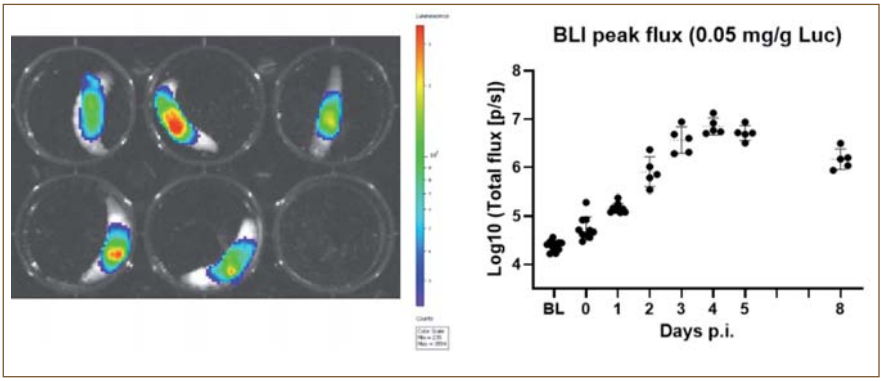

Figure 2. BLI of *Aspergillus fumigatus* fungal load in *Galleria mellonella* larvae over time using a 0.05 mg/g D-luciferin dose. 10 larvae were infected with 1 x 105 spores AF on day 0 and were daily injected with 0.05 mg/g D-luciferin. A. Bioluminescence visualization of AF infected larvae at day 4 post infection (p.i.). B. Quantification of BLI peak flux over time from baseline (BL) until day 8 p.i. as an objective readout of AF fungal load in *G. mellonella* larvae.

lity of BLI as an objective and dynamic readout of fungal load in *G. mellonella.* Further optimization will focus on testing lower doses of D-luciferin to reduce toxicity while maximizing BLI signal to efficiently quantify the fungal load.

Conclusion. These results suggest that BLI is a feasible readout to quantify AF in *G. mellonella* larvae over time using a concentration of 0.05 mg/g D-luciferin. However, since daily D-luciferin injection decreased larval survival, a lower luciferin dose needs to be defined to reduce toxicity while retaining sufficient BLI signal. This optimized BLI-compatible *G. mellonella* model will enable objective *in vivo* screening of antifungal efficacy against infection with azole-resistant and -susceptible AF strains, ultimately facilitating a more ethical and cost-effective translation of promising antifungal drugs towards mammalian models.

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OC7

GALLERIA MELLONELLA LARVAE AS AN EFFICIENT *IN VIVO* MODEL TO CHARACTERIZE THE EFFICACY OF ANTIMICROBIAL PEPTIDES AGAINST MULTI-DRUG RESISTANT ENTEROAGGREGATIVE *E. COLI*

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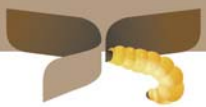
Background: Drug discovery is warranted for the identification of effective therapeutic candidates to overcome antimicrobial resistance. The identified molecules need to be screened in high throughput laboratory models for their efficacy; *Galleria mellonella* larvae is one among them. Hence, the present study was undertaken to evaluate the in vivo antimicrobial efficacy of three short-chain cationic antimicrobial peptides (AMPs) against multi-drug resistant enteroaggregative Escherichia coli (MDR-EAEC).
Methods: *In vitro* minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of AMPs (indolicidin, CAMA, and lactoferricin [17-30]) were determined by broth micro-dilution technique. Prior to *in vivo* study, the AMPs were tested for their *in vitro* stability, safety, adverse effect on beneficial lactobacilli, membrane permeability assays, and dose- and time-dependent time-kill assays. Further, the *in vivo* antimicrobial efficacy of individual AMPs was performed in *G. mellonella* larval model employing survival assay, bacterial burden, haemocyte density, melanisation assay, cytotoxicity assay, and histopathological examination.
Results: MIC values (µM) observed for indolicidin, CAMA and lactoferricin (17-30) were 32.0, 2.0 and 32.0, respectively, whereas the MBCs were either equal to or twice greater than the MIC values. All the three AMPs were found stable, tested safe at MIC value with no adverse ef-



fect against beneficial lactobacilli. AMPs exhibited membrane permeability in a dose- and time-dependent manner. In vitro time- kill assay revealed concentration- cum- time-dependent clearance of MDR-EAEC in the AMP-treated groups, while, in the *in vivo G. mellonella* experiment, the infected group treated with AMPs revealed an improved survival rate, immunomodulatory effect, reduced MDR-EAEC counts, and were tested safe to the larval cells which concurred with histopathological observations. The AMPs exhibited either an equal or better efficacy than the tested antibiotic control, meropenem.

Conclusion: This study highlights the possibility of *G. mellonella* larvae as an excellent *in vivo* model for investigating the efficacy of antimicrobial peptides against MDR-EAEC strains.

OC12

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* USA300 PERSISTER CELLS SHOW CHAPERONE UPREGULATION IN CONTRAST TO PLANKTONIC CELLS AND THE BIOFILM PHENOTYPE

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Background: Bacteria produce biofilms – bacterial communities embedded in an extracellular matrix that protects the organisms from antibiotics and the host immune response – and persisters (PS) – cells that neither grow nor die in the presence of bactericidal agents, thus exhibiting multidrug tolerance. We developed distinct models to isolate PS and biofilms formed by methicillin-resistant *Staphylococcus aureus* USA300 (MRSA-USA300), a highly virulent clone that causes fulminant infections. Here, we compared the transcriptomic profiles of MRSA-USA300 biofilms and PS against planktonic (PL) cells.
Materials/methods: MRSA-USA300 biofilm was grown in brain heart infusion (BHI) medium with 0.1% glucose under static conditions for 24h, 48h and 72h. PS were isolated by treating a 16h stationary phase culture with 5 µg/ml ciprofloxacin for 24h. Samples were taken at 0h, 0.5h, 1h, 2h, 4h, 6h, 8h and 24h, washed and plated for viable cell count. As a comparator, PL cultures were generated in parallel in both cases. Total RNA-extraction (MasterPure™Complete DNA/RNA Purification Kit, Lucigen), rRNA depletion (Ribo-Zero rRNA Removal Kit (Gram-Positive Bacteria), and RNA-seq (NextSeq, Illumina) were performed on all samples. Differential-gene-expression analysis was performed using DESeq2 with log2 fold change (FC) > 1 or < -1 and $p \leq 0.05$ considered significant.
Results: MRSA-USA300-PS were successfully generated after 24h ciprofloxacin treatment, typified by a biphasic killing curve (Figure A). Components of chaperone-complex dnaK-dnaJ-grpE and complemen-

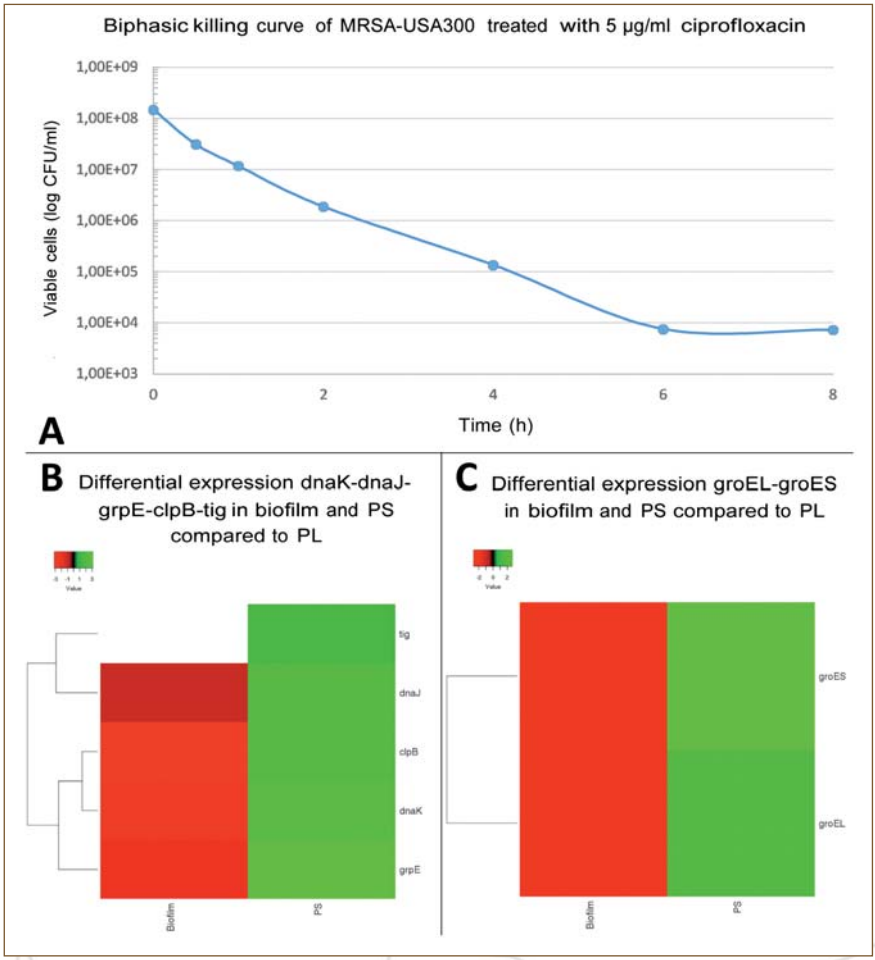
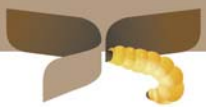


Figure: A) When treating MRSA-USA300 with 5µg/ml ciprofloxacin, the killing curve has a biphasic nature. The susceptible cells die faster in the earlier stages of antibiotic treatment whilst the killing plateaus at 6-8h indicating that, at this stage, only PS form a major part of the bacterial population. 0h equals start of antibiotic treatment of a 16h stationary culture. B) Differential expression analysis of dnaK-dnaJ-grpE-clpB-tig shows significant upregulation (log_2 FC > 1; green) in PS compared to PL but contrasting downregulation (log_2 FC < -1; red) in biofilm compared to PL. tig did not show significant differential expression in biofilm (white). (dnaK: Chaperone protein DnaK – dnaJ: Chaperone protein DnaJ – grpE: Nucleotide exchange factor GrpE – clpB: Chaperone protein ClpB – tig: Trigger factor Tig) C) Additionally, differential expression analysis of groEL-groES shows significant upregulation (log2 FC > 1; green) in PS compared to PL but contrasting downregulation (log2 FC < -1; red) in biofilm compared to PL.

tary genes clpB and tig showed significant upregulation (log_2 FC: 2.47, 2.08, 3.15, 2.14 and 1.32 respectively) in PS compared to PL ($p \leq 0.001$). In contrast, significant downregulation of said genes, except tip ($p > 0.05$), was observed in biofilm (log_2 FC: -2.72, -1.13, -1.95 and -3.10 respectively) compared to PL ($p \leq 0.024$) (Figure B). In addition, chaperone-complex groEL-groES gene showed significant upregulation in PS (log_2 FC: 1.70 and 2.70 respectively; $p \leq 0.001$) and again contrasting significant downregulation in biofilm (log_2 FC: -2.01 and -1.98 respectively; $p \leq 0.004$) compared to PL (Figure C).

Conclusions: Our data shows that MRSA-USA300-PS upregulate chaperone-complexes, in contrast to the biofilm phenotype, as was previously suggested for *E. coli* PS. Upregulated chaperone expression aids in preventing protein misfolding and aggregation under stress conditions. Increased activity would also lead to ATP-depletion, a known feature of PS.



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