RST – Extended scientific and tehnical raport

Project title: Transcriptomic aspect of host-pathogen-antibiotic interations in *Drosophila melanogaster* infectet with ESKAPE pathogens (TRANSCEND)

code PN-III-P4-PCE-2021-1797, contract 96/2022

RST – extended scientific and tehnical summary - phase II

Objectives and activities:

Ob2. Comparative evaluation of *in vitro* and *in vivo* variable expression of the specific antibiotic resistance genes

2.1 Performing the infection experiments in optimized conditions, regarding the bacterial inoculums density, the infection dose, antibiotic dose, the duration of the experimental infection

2.2 Cultivation of bacterial strains on artificial media, with and without antibiotic

2.3 qRT-PCR assessement of the targeted genes from resistant pathogens genomes

2.4 Evaluation of *D. melanogaster* microbiota changes using a culture-independent assay during infection and/or antobiotic treatment

2.5 Data analysis and interpretation

Ob3. Revealing particular eukaryotic genes expression profiles in infected *versus* uninfected fruit flies, either in the absence or in the presence of experimental colistin treatment

3.1 Achieving the infection experiments in the optimized conditions

3.2 Fulfil qRT-PCR for the target genes from *D. melanogaster*

3.3 Data analysis and interpretation

Phase II summary

All planned activities in the second phase of the project have been succesfully performed thus fulfilling the project's objectives. Experimental procedures consisted of *D. melanogaster* experimental infection and/or antibiotic treatment in optimized conditions, recovery of antibiotic resistant bacterial strains, qRT-PCR gene expression evaluation on either pathogen genes of *D. melanogaster* targeted genes, and microbiota profiles analysis using culture-independent techniques. The workflow of this experimental phase is presented in Figure 1.

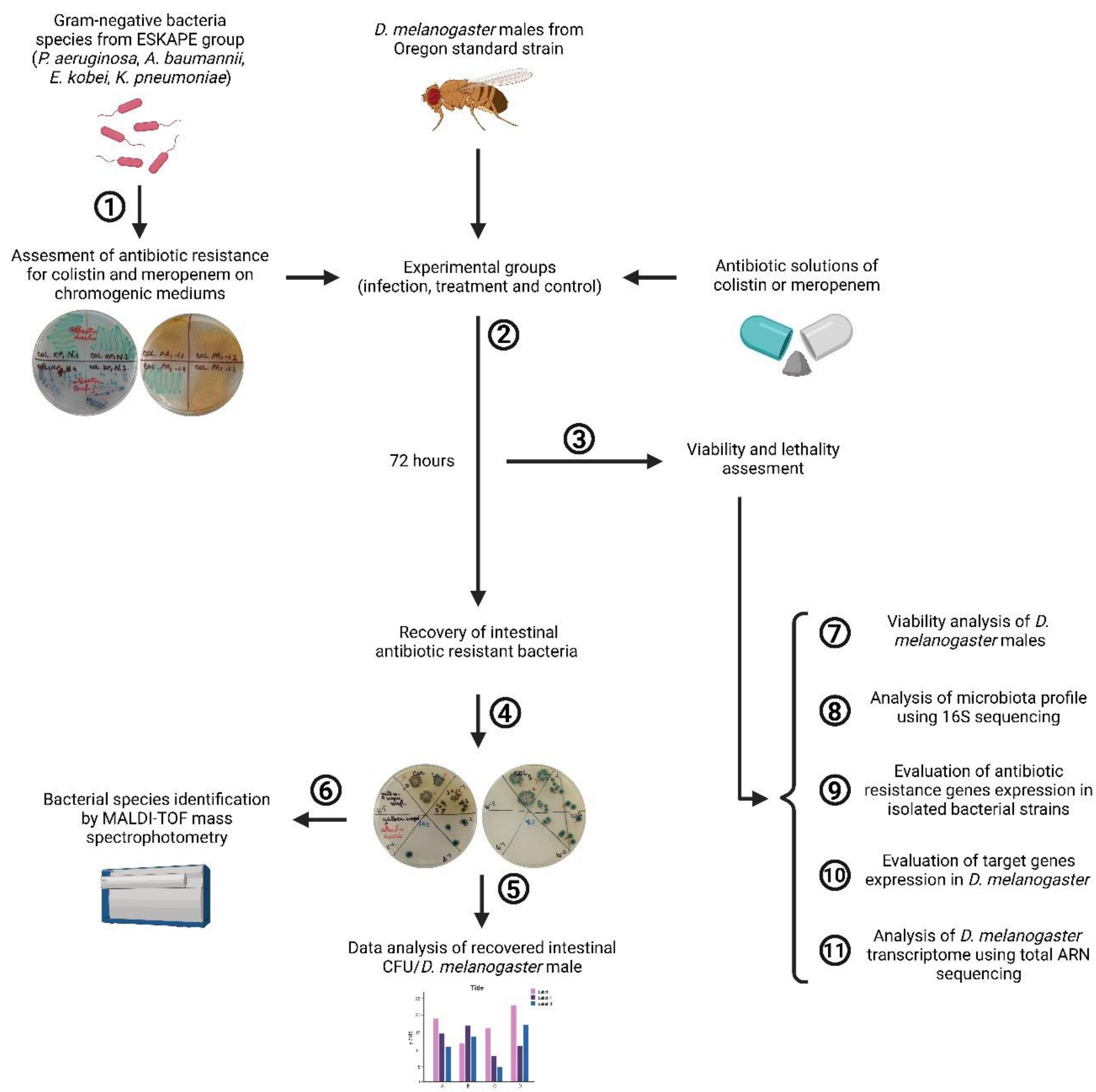

Figure 1. Graphic representation of expeirmental workflow in Phase II of the project.

In phase II experimental activities have been performed on *D. melanogaster* males pertaining from three lines with distinct genotypes, namely the standard control Oregon, mutant heterozygotes for *γCop* gene ($\gamma COP^{S057302}$/+), and mutant homozygotes for the same gene ($\gamma COP^{14a}/\gamma COP^{14a}$). All individuals were tested in different experimental conditions: i) control, ii) infection with either *Enterobacter kobei* or *Acinetobacter baumannii*, iii) infection + treatment with colistin/meropenem. A bacterial inoculum of 4,5 $\times 10^9$ colony forming units (CFU) suspended in 5% sucrose solution has been used for infection by pipetting on sterile filter paper. The control experimental group has only been adimistered a 5% sucrose solution.

Last resort antibiotic treatment with colisin or meropenem resulted in recovery of *Providencia rettgeri* yellow colonies on COL-APSE agar, after 18 hours of treatment. Following infection with *A. baumannii*, antibiotic resistant antibiotics have been recovered for all experimental conditions, while in case of *E. kobei* infection, the resistant colonies of the infecting strain have been recovered on mSuperCARBA agar for all experimental conditions and *P. rettgeri* yellow colonies on COL-APSE.

Bacterial genes expression evaluation targeted *KPC* and *CTX-M* resistance genes in *D. melanogaster* males infected with *E. kobei* in the absence or presence of colinstin treatment. Overexpression of these genes has been observed following *E. kobei* infection when compared to the infection + treatment condition.

Prior to selecting specific genes of interest in *D. melanogaster* for qRT-PCR, highthroughput screening using RNA-sequencing has been performed. Preliminary data revealed that *Relish* and *dorsal* were adequate candidates for targeted gene expression. qRT-PCR has been performed on *D. melanogaster* $\gamma COP^{S057302}$/+ mutant males infected with *E. kobei* and treated with colistin. Data analysis showed overexpression in *Relish* and *dorsal* genes following infection with *E. kobei* compared to control, and underexpression following infection + treatment compared to control.

Microbiota profiles of *D. melanogaster* males infected with *A. baumannii* and/or treated with antibiotics were assesed by performing ARNr 16S sequencing, a novel technique implemented in Romanian research. Experimental data showed significant changes in commensal microbiota profiles in *D. melanogaster* males mutant for *γCop* gene, the mutant background being associated with a significantly higher abundance of Gram-negative, pro-inflammatory microbiota.

Results dissemination

Experimental results have been presented by publishing three ISI original articles, two BDI original articles, and by participating in international conferences, as well as national conferences and workshops. The project's web page has been updated with results generated in the second experimental phase.

ISI articles

1. Barbu, I.C.; Gheorghe-Barbu, I.; Grigore, G.A.; Vrancianu, C.O.; Chifiriuc, M.C. Antimicrobial Resistance in Romania: Updates on Gram-Negative ESCAPE Pathogens in the Clinical, Veterinary, and Aquatic Sectors. *Int. J. Mol. Sci.* **2023**, *24*, 7892. https://doi.org/10.3390/ijms24097892.
2. Truşcă, B.S.; Gheorghe-Barbu, I.; Manea, M.; Ianculescu, E.; Barbu, I.C.; Măruțescu, L.G.; Dițu, L.-M.; Chifiriuc, M.-C.; Lazăr, V. Snapshot of Phenotypic and Molecular Virulence and Resistance Profiles in Multidrug-Resistant Strains Isolated in a Tertiary Hospital in Romania. *Pathogens* **2023**, *12*, 609. https://doi.org/10.3390/pathogens12040609.
3. Ionascu, A.; Ecovoiu, A.A; Chifiriuc, M.C.; Ratiu, A.C. qDATA - an R application implementing a practical framework for analyzing quantitative Real-Time PCR data. In Press.

BDI articles

1. Vrâncianu, C.O.; Gheorghe-Barbu, I.; Czobor Barbu, I.; Mãruțescu, L.; Popa, M.; Nițã-Lazãr, M.; Muntean, A.A.; Dragomirescu, C.; Sãndulescu, O.; Talapan, D.; Sidoroff, M.E.; Tusa, I.M.; Popa, M.I.; Chifiriuc, M.C. Antibiotic resistance profiles in *Acinetobacter Baumannii* strains isolated from wastewater in southern Romania. *Romanian archives of microbiology and immunology* **2022**, 81 (4) 257-263.

International conferences

1. Muntean A.; Chifiriuc M.C. E*volution and implications of antibiotic resistance in veterinary and* environmental *sectors - key issues for a One Health approach* Int. Conference One Health-One Earth, 24-25.11. 2023, Cluj Napoca, Romania

National conferences and workshops

1. Chifiriuc, M.C. Antimicrobial drug resistance – research directions at the Faculty of Biology. ZIM, Bucuresti, 18 Sept. 2023
2. Chifiriuc, M.C. Roles of microbiota in oncologic patient management. Interacțiuni interdisciplinare la frontiera medicinii personalizate. SMART DIASPORA, Timișoara, Apr. 2023
3. Chifiriuc, M.C. Omul și microbiomul: între Eros și Thanatos. Noi frontiere și provocări ale abordărilor transdisciplinare – Analiza și Controlul Dinamicii Sistemelor Celulare. SMART DIASPORA, Timișoara, Apr. 2023
4. Constantin, N.D.; Ionașcu, A.; Rațiu, A.C.; Chifiriuc, M.C.; Barbu, I.C.; Csutak, O.; Ecovoiu, Al.A. Analiza răspunsurilor fiziologice la infecții bacteriene și tratament cu meropenem ale masculilor din linia Oregon de Drosophila melanogaster. Sesiunea de Comunicări Științifice a Studenților Facultății de Biologie, București, 12 mai 2023.

5. Ionașcu, A.; Constantin, N.D.; Rațiu, A.C.; Chifiriuc, M.C.; Barbu, I.C.; Gheorghe-Barbu, I.; Ecovoiu, Al.A. Evaluarea prin metode dependente de cultivare a încărcăturii microbioene la masculi de D. melanogaster infectați cu tulpini patogene multirezistente sau tratați cu colistin. Sesiunea de Comunicări Științifice a Studenților Facultății de Biologie, București, 12 Mai 2023.
6. Chifiriuc, M.C.; Ionașcu, A.; Constantin, N.D.; Rațiu, A.C.; Gheorghe-Barbu, I.; Barbu, I.C.; Ecovoiu, Al.A. Experimental setup optimizations for evaluating microbiome antibiotic interactions in D. melanogaster model. CIVIS Summer School, Drug Design and Discovery, București, 7 Iulie 2023.
7. Rațiu A.C.; Ecovoiu A. Studii de genomică și bioinformatică pe modelul experimental *Drosophila melanogaster*, Prima ediție a Conferinței Anuale de Comunicare a Rezultatelor Cercetării la Universitatea din București, 23-24.02.2023
8. Constantin, N.D.; Ionașcu, A.; Barbu, I.C.; Ecovoiu, Al.A.; Gheorghe-Barbu, I.; Grădișteanu Pircalabioru, G.; Rațiu, A.C.; Chifiriuc, M.C. Utilizarea Drosophila melanogaster pentru modelarea experimentală a unor infecții bacteriene. Conferința Anuală de Comunicare a Rezultatelor Cercetării la Universitatea din București, 17 Noiembrie 2023.