Phase II Summary

Metagenomic analyses of aquatic and fish microbiota for antibiotic resistance genes (ARGs) virulence markers (VMs) and plasmids, highlighting the connections between environmental and fish microbiota-sourced ARGs/VMs/plasmids.

Continuation of stage I - Comparative phenotypic and molecular characterization of MDR CP ESBL AB circulating clones, isolated for 4 years consecutively from clinical and aquatic environment compartments [wastewater network (hospital sewage, wastewater treatment plant) and the receiving river] and demonstrate *in vitro* the transfer of antimicrobial resistance markers from bacteria isolated from hospital/sewage to bacteria occurring in receiving river

Reported period: 01.01.2023-31.12.2023

The second stage of the project aim to assess: i) the metagenomic analyses of aquatic and fish microbiota for antibiotic resistance genes (ARGs), virulence markers (VMs) and plasmids, highlighting the connections between environmental and fish microbiota-sourced ARGs/VMs/plasmids; ii) the comparative phenotypic and molecular characterization of MDR CP ESBL AB circulating clones, isolated for 4 years consecutively from clinical and aquatic environment compartments [wastewater network (hospital sewage, wastewater treatment plant) and the receiving river] and iii) demonstrate *in vitro* the horizontal transfer of antimicrobial resistance markers from bacteria isolated from hospital/sewage to bacteria occurring in receiving river. For this purpose, 8 surface water samples and fish samples collected from the upstream and downstream regions of urban WWTPs from 3 representative regions for Romania were subjected to metagenomic sequencing. Clonal relationships of *Acinetobacter baumannii* strains were investigated by molecular typing methods (ERIC-PCR, *Whole Genome Sequencing, MultiLocusSequence Typing*), chromosomal or plasmid location of carbapenem resistance genes (CRG) was demonstrated by 3rd generation sequencing and transferability of OXA-23 and OXA-24 carbapenemases (CP) by conjugation using as donors *A. baumannii* strains selected strains and a rifampicin-resistant *Acinetobacter baylyi* strain as recipient. The aquatic microbiota is represented by *Bacteroidetes* (16%), *Betaproteobacteria* (16%), *Gammaproteobacteria* (14%), *Firmicutes* (11%), *Alphaproteobacteria* (10%), *Actinobacteria* (9%) and *Deltaproteobacteria* (5%), and with a lower frequency *Thermodesulfobacteria*, *Deferribacteres*, *Fibrobacteres* and *Elusimicrobia* taxa were identified. The fish microbiota samples collected from the Ialomița/Dâmbovița rivers was represented by of *Aeromonadales* (28%/56%); *Fusobacteriaceae* (0/15%); *Flavobacteriaceae* (10%/0%); *Enterobacteriaceae* (5%/8%) and *Vibrionaceae* (4%) species. A high diversity of ARGs was identified in the surface water samples microbiota, being demonstrated the presence of extended spectrum β-lactamases encoding genes (TEM-126, TEM-1A, TEM-34, TLA-1, VEB-1, VEB-9, BEL-1, CARB-5), cephalosporinases (CMY-12, CMY-4, MOX-3, MOX-9), carbapenemases (IMP-33, GES-5) and oxacillinases (OXA-1, -2, -4, -5, -10, -12, -13, -15, -16, -20, -37, -58, -129, -205,-256, -320, -347, -504, -534, -539, -540, -732, -827). Aminoglycosides, macrolides, phenicols, quinolones, sulfonamides, tetracyclines and polymyxins ARGs were identified in the microbiota samples collected from all isolation sources and locations with the exception of the water sample collected from the Bahlui river (the upstream region of WWTP Iași). Biocide resistance genes, heavy metals (As, Cr, Cu, Hg, Mn, Ag, Zn, Co) and VMs involved in the adherence, biofilm formation, modulation of the host immune response, metabolism, regulatory processes, etc. were identified in all isolate sources. A total of 2107 *IS*, 2002 *ICE* (17 conjugative plasmids, 94% identified in the microbiota of water sample from the downstream region of WWTP Bucharest) and 588 T*n* were associated with the microbiota of the investigated sources. Molecular typing of *A. baumannii* strains isolated during 2019-2022 from intra-hospital infections, wastewater and surface water samples in Romania demonstrated that strains belonged to a total of 45 ERIC-PCR profiles, 39 circulating clones between the hospital settings, wastewater and surface water network and respectively to 4 high-risk international clones (IC2; IC1; IC5 IC8). Through third-generation sequencing techniques, the chromosomal or plasmid location of CRG $bla_{\text{OXA-23}}$ (pA105-like) and, respectively, $bla_{\text{OXA-72}}$ (pMAL1) was demonstrated in *A. baumannii* strains belonging to high-risk clones. Conjugation experiments demonstrated the transfer of OXA-23 and OXA-24 CP from donors to recipient with transfer rates - 1.9×10^{-7} - 3.3×10^{-13} / 2.5×10^{-6} - 3×10^{-12} / 1.9×10^{-9} – 1.8×10^{-11} in the South/North-East/Center-West regions. Therefore, all planned activities have been full completed, and the result indicators have been achieved or even exceeded. The dissemination of the results was achieved through the permanent updating of the project website, the participation with oral presentations (3) and posters (3) at two

international and one national conferences and the publication of 4 scientific papers [article (2) and review (2) type) in ISI journals which mention the project in the Acknowledgment section.

Results dissemination

Experimental results have been presented by publishing four ISI papers (article and review type), and by participating in international conferences with 4 oral presentations (2) and paper posters (2) at the 18th International Conference on Chemistry and the Environment (ICCE 2023), Venice, Italy, June 11-15, 2023, paper poster at the 8th International Conference on Clinical Metagenomics, Geneva, Switzerland, 16-17.11.2023, as well as at national conferences - International Day of Microorganisms, 3rd Ed. 18 September 2023, Faculty of Biology, Bucharest.

ISI articles

- Gheorghe-Barbu I., Corbu V.M. Vrancianu C.O. Marinas I.C. Popa M. Dumbravă A.Ș. Niță-Lazăr M. Pecete I. Muntean, A.A.; Popa, M.I.; et al. Phenotypic and Genotypic Characterization of Recently Isolated Multidrug-Resistant *Acinetobacter baumannii* Clinical and Aquatic Strains and Demonstration of Silver Nanoparticle Potency. *Microorganisms* 2023, 11, 2439. https://www.mdpi.com/2076-2607/11/10/2439.
- Czobor 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://www.mdpi.com/1422-0067/24/9/7892.
- Trușcă 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://www.mdpi.com/2076- 0817/12/4/609.
- Vrâncianu C.O., Serban B., Gheorghe-Barbu I., Czobor Barbu, I., Cristian, R.E., Chifiriuc, M.C., Cirstoiu, C. The Challenge of Periprosthetic Joint Infection Diagnosis: From Current Methods to Emerging Biomarkers. *Int. J. Mol. Sci*. 2023, 24, 4320. https://www.mdpi.com/1422-0067/24/5/4320.

International conferences

- Surleac M., Czobor Barbu I., Paraschiv S., Gheorghe-Barbu I., Oțelea D., Chifiriuc M.C. Bioinformatic Insights into Resistant *Escherichia Coli* Isolated from Different Aquatic Environments in Romania. 18th International Conference on Chemistry and the Environment (ICCE 2023), oral presentation OP404, 11 - 15 iunie 2023, Venice, Italy.
- Gheorghe-Barbu I. Czobor Barbu I., V.M. Corbu, G.A. Grigore, O.C. Vrâncianu, M. Surleac, S. Paraschiv, L. Bănică, A.M. Muntean, I. Pecete, L. Măruțescu, M. Popa, M.I. Popa, D. Oțelea, M.C. Chifiriuc. Comparative Phenotypic and Molecular Characterization of Clinical and Aquatic Multidrug Resistant *Acinetobacter baumannii* Circulating Clones Isolated in Romania for Four Consecutive Years. 18th International Conference on Chemistry and the Environment (ICCE 2023), oral presentation OP225, 11 - 15 iunie 2023, Venice, Italy.
- Barbu I.C., Gheorghe-Barbu I., Surleac M., Vlaicu O., Paraschiv S., Rațiu A., Chifiriuc M.C. Clonal Transmission of Colistin Resistant *Klebsiella Pneumoniae* Strains Harboring Endemic Mutations in *Mgrb* Gene in Clinical Settings and Wastewaters from Romania. 18th 18th International Conference on Chemistry and the Environment (ICCE 2023), Poster: PP2_018, 11 - 15 iunie 2023, Venice, Italy.
- Gheorghe-Barbu I., Czobor Barbu I., Corbu V.M., Dumbravă A.Ș., Surleac M., Paraschiv S., Oțelea D., Marinescu L., Ficai D., Ficai A., Chifiriuc M.C. *In Vitro* Evaluation of Silver Nanoparticles Activity Against 132 Multidrug Resistant *Acinetobacter baumannii* Strains Isolated From Romanian Hospitals and Aquatic Environments During 2019–2022. 18th International Conference on Chemistry and the Environment (ICCE 2023), Poster: PP2_017, 11 - 15 iunie 2023, Venice, Italy.
- Surleac M., Barbu I., Paraschiv S., Gheorghe-Barbu I., Oțelea D., Chifiriuc M.C. Antimicrobial resistance, MGEs and functional profiling through use of shotgun metagenomics on isolates from receiving surface waters proximal to WWTPs. Poster - 8th International Conference on Clinical Metagenomics, 16-17.11.2023, Geneva, Switzerland.

National conferences

- Gheorghe-Barbu I., Czobor Barbu I., Corbu V.M., Vrâncianu O.C., Marinas I.C., Dumbrava A.S., Surleac M., Paraschiv S., Muntean A.A., Marinescu L., Ficai D., Ficai A., Măruțescu L., Popa M., Nita Lazar M., Popa M.I., Dan Oțelea D., Chifiriuc M.C. Caracterizarea fenotipică și moleculară a clonelor multirezistente de *Acinetobacter baumannii* circulante în infecții intraspitalicești și mediul acvatic din România patru ani consecutiv și demonstrarea eficientei antimicrobiene a nanoparticulelor de argint.

Ziua Internationala a Microorganismelor, Editia a III-a 18 septembrie 2023, Facultatea de Biologie, Bucuresti.