

Phase I Summary

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] Reported period: 13/05/2022 - 31/12/2022

The first stage of the project aim to assess the phenotypic and molecular investigation of β -lactam resistance in *Acinetobacter baumannii* (AB) strains isolated and identified 4 years consecutively from intra-hospital infections and different aquatic environment compartments (wastewater and surface water network) from 3 geographical regions representative of Romania, to determine the carbapenemase (CP), respectively the extended spectrum β -lactamases (ESBL) type and to and to highlight genetic virulence markers (VM) in multidrug-resistant strains (MDR) CP respectively ESBL producing strains. For this, between 19.07.2022 - 26.08.2022, wastewater and surface water samples were collected, the total microbial load corresponding to AB species was determined on the CHROMagar *Acinetobacter* chromogenic medium, respectively the microbial load corresponding to non-fermenting Gram negative bacilli (BGNNF) on chromogenic media supplemented with antibiotics (CHROMagar CARBA, CHROMagar ESBL and CHROMagar Colistin); isolated AB strains were presumptively identified and confirmed by MALDI-TOF mass spectrometry. The antibiotic resistance profiles of the AB strains isolated in 2022 were compared with those previously isolated (2019-2021) from the same isolation sources and geographical locations and the VMs were investigated at the genotypic level. During this phase a total number of 30 wastewater and surface water samples from 7 geographical locations of Romania which allowed the identification of a total number of 135 AB strains, the highest isolation frequency being associated with water samples collected from large cities, county seat [Bucharest, Târgoviște (24.44%); Iasi (20.74%)] and respectively Galati (18.51%), a city located on the lower course of the Danube river, considered the future central axis of the European Union. In the same temporal sequence 8 strains corresponding to AB species were isolated from intra-hospital infections. The comparative analysis of the antibiotic resistance (AR) profiles according to the isolation source and the geographical location of a total number of 587 AB strains isolated during 2019-2022 demonstrated comparable levels: the strains isolated from intra-hospital infections before pandemic / after-pandemic period showed the highest resistance level to CR (IMP, MEM) in all 3 geographical regions; in the case of the strains isolated from the analysed WWTPs samples, the CR resistance level varied, thus for the Southern region in Bucharest: 41.1%/64.7% and respectively 12.12%/18.18%; Vâlcea: 7.14%/7.14% and respectively 0%/0%; Târgoviste: 94.4%/92.5% and respectively 0%/100%. For the central and western region, lower levels of AR were identified: Cluj: 0%/0% and respectively 0%/50%; Timișoara: 50%/50% and respectively 0%/0%, and for the North and Eastern regions the CR levels varied: from 50%/100% and respectively 42.8%/94.9% in Iași to 78.1%/78.1% and respectively 33.3% /33.3% in Galați. The genotypic study of CR and cephalosporin resistance demonstrated the presence of CPs OXA-66, OXA-23, OXA-72 and ESBLs TEM, SHV, CTX-M, PER, VEB, GES circulating between the hospitals, wastewater and surface water network; the absence of acquired CP and ESBL encoding genes in the case of AB strains isolated in 2022 from wastewater and surface water samples being associated with the presence of VMs (*ompA*, *csuE* and *bfmS*).