

The emergence and spread of antibiotic resistance among bacterial pathogens is the most striking example of evolution that has been observed in bacteria over the past seventy years.

Intensive and uncontrolled use of antibiotics not only therapeutically in human and veterinary medicine, but also in animal breeding and agriculture, have provoked the worldwide spread of highly resistant bacteria. A serious consequence of antibiotics misuse is the phenomenon of propagation of resistance genes by horizontal gene transfer, HGT. The presence of antibiotics in the environment in concentrations lower than those inhibiting bacterial growth, causes not only selection of resistant strains but also an increase in frequency of HGT between strains of the same or even different species. At the beginning bacterial resistance was noted in hospital setting, but now multidrug resistant strains, i.e. showing resistance to more than two unrelated antimicrobials, are isolated more and more often from the environment.

Recent studies have shown that resistance integrons are responsible mainly for the emergence and spread of multidrug antibiotic resistance among bacterial pathogens. The integron is a DNA fragment that is capable of capturing gene cassettes coding for antibiotic resistance. As the integron system has the ability to create novel combinations of resistance genes, it may facilitate the appearance of multidrug resistant bacteria. It is essential that, as integrons are embedded in mobile genetic elements, they can be transferred within a microbial population through HGT.

Water environment, especially urban wastewater treatment plant (WWTPs), creates excellent conditions for spreading of resistant bacteria and resistance integrons. The features of this environment, like the high number of bacteria originating from different sources, nutrients and the presence of trace concentrations of antibiotics, may significantly facilitate the transfer of mobile genetic elements, which leads to multiplication of resistance genes and emergence of multidrug resistant strains in the effluent. The effluent, which is discharged to the river water, creates repository of the most threatening bacteria - those producing extended-spectrum β -lactamases (ESBL), carbapenemases, metallo- β -lactamases and strains resistant to vancomycin and methicillin. It has been proved that bacteria producing extended spectrum β -lactamases and carbapenemases are resistant to all β -lactam antimicrobials used in the treatment of Gram-negative bacterial infections. Hence, with their key position at the interface between human activities and the environment, WWTPs can be used to control antimicrobial resistance.

The aim of the project is to evaluate the occurrence and frequency of significant antibiotic resistance genes (ARGs) and resistance integrons in the genomes of culturable bacteria and in the wastewater metagenome of Central Wastewater Treatment Plant in Kozięłowy near Poznań. "Traditional" approach based on analysis of DNA isolated from bacteria cultured from wastewater samples is limited to certain groups of microorganisms and does not take into consideration viable but non-culturable bacteria that can make up even 99% of all microorganisms present in sample. Quantitative metagenomic analysis combined with next-generation sequencing would allow determining not only the dynamics of changes in the abundance and type of ARGs and integrons, but also the related changes in the bacterial population in consecutive stages of wastewater treatment.

This approach will allow:

- Determination of presence and type of ARGs and integrons in the genomes of culturable bacteria isolated from sewage at different stages of water treatment
- Determination of copy number and abundance of ARGs and integrons integrase genes in wastewater metagenome.
- Analysis of sequence diversity of ARGs significant for public health in order to determine variants of genes present in the metagenome and the genomes of culturable bacteria.
- Determination of genetic content of integrons present in the genomes of culturable bacteria.
- Metagenomic analysis of the composition of bacterial populations in the wastewater.

The results of the project would provide knowledge on the dynamics of changes in the copy number and abundance of antibiotic resistance genes and integrons, and changes in the bacterial population; moreover, they would allow determining the conduits of resistance spread and the participation of wastewater effluent in the extension of environmental resistome. Understanding sources and mechanisms of antibiotic resistance genes dissemination in water environment is critical for developing effective strategies for containing antibiotic resistance.