

In recent years antibiotics, antibiotic resistant bacteria and antibiotic resistance genes have been identified in almost all segments of the environments and perceived as the main environmental contaminants. For many years antibiotic resistance studies have mainly focused on breeding and indicator organisms. As it is generally known, most microorganisms cannot be identified with breeding methods. In addition, there is still little information on the spread of antibiotic resistance genes and antibiotic resistant bacteria in different environments.

Wastewater treatment systems (wastewater from hospitals, pharmaceutical industry, as well as household and municipal sewage) are regarded as the main emitters of not only antibiotics but also antibiotic resistant microbes and antibiotic resistance genes. Wastewater treatment plants are considered to be reservoirs of resistance determinants. Treated wastewater, which has so far been perceived as safe for the environment, contains a large load of determinants of antibiotic resistance. Ineffective removal of antibiotic resistance determinants is a serious environmental and health problem, although still invisible to the naked eye (as opposed to e.g. suspensions or biogenic elements causing eutrophication of water bodies).

Furthermore, after the penetration of wastewater containing antibiotic resistant strains and antibiotic resistance genes the role of resistance reservoir can be and is taken over by environmental strains that acquire virulence properties and consequently become resistant and pathogenic. Considering the unquestionable health threat posed by antibiotic resistance and its spread in wastewater treatment plants, the current project is part of a contemporary research trend focused on understanding the coexistence of antibiotic resistance genes and mobile elements among environmental microbiota. The submitted project includes research based on a metagenomic analysis as a modern microbiological method, explaining the diversity and spread of antibiotic resistance within wastewater treatment plants as major emitters of this type of pollutants and their receivers. In the study metagenomic approach. Illumina high-throughput sequencing (HTS) will be used for profiling ARGs and MGEs. To evaluate the obtain results large-scale bioinformatic approach based on (taxonomic, functional, antibiotic resistance and integrase genes) using modern bioinformatics programs will be applied.

Under the submitted project tests on the presence of ARGs-antibiotic resistance genes from the following groups: β -lactams, quinolones, sulphonamides, macrolides and tetracyclines, and their coexistence with mobile genetic elements (MGEs), mainly genes of integrase (*int11*, *int12*, *int13*) will be carried out based on the metagenomic profile. The subject of the research will be wastewater from two municipal wastewater treatment plants and surface water - receivers of purified sewage. Most of the antibiotic resistance genes are located in the DNA of mobile genetic elements, such as, for example: transposons, integrons and plasmids. Mobile determinants of resistance can move within the genome or between the genomes of different prokaryotes.

The scientific aim of the project is to investigate and explain the coexistence of antibiotic resistance genes and mobile genetic elements, mainly the integrase genes (*int11*, *int12*, *int13*) in wastewater and water in receivers using a metagenomics technique. The basic scientific research that is planned to be carried out under this project will provide new knowledge about the spread of antibiotic resistance genes and mobile elements - integrons. Metagenomic analysis will enable detection of the investigated genes in the microbial communities in wastewater from two treatment plants and sewage receivers. The research will establish the relationship between the antibiotic resistance genes and the *int11*, *int12*, *int13* genes. During the project implementation, it will be particularly important to determine how the antibiotic resistance genes and the mobile genetic elements affect the propagation and changes of these genes in the receiver's water. Knowledge about the formation and spread of antibiotic resistance genes will be very helpful in understanding the environmental resistomes.

The experimental basic research realized in proposed project concerning prevalence of antibiotic resistance genes and their mobile elements will provide new knowledge on the comprehensive insight into microbial community and functions of wastewater and receiving water. Metagenomic analysis used will reveal of a variety and simultaneous detection of ARGs and MGEs in microbial communities in both WWTP and in receiving waters.

The project is implemented by a team of microbiologists and biochemists from the Institute for Ecology of Industrial Areas in Katowice and specialists in molecular biology, sanitary engineering and bioinformatics from the University of Warmia and Mazury in Olsztyn.