

The widespread use of antibiotics, both in human treatment and in agriculture and animal husbandry, has led to the increasing occurrence of microbial resistance. The concept of "One Health" promoted by the World Health Organization, emphasizes the need for close collaboration between human health, animal health, and condition of environment. Wastewater treatment plants provide excellent conditions for the spread of resistant bacteria in the environment, along with the discharged sewage, sewage sludge used for fertilization, and generated bioaerosols. A particularly dangerous phenomenon is the emergence of resistance to new drugs and so-called "last-resort" antibiotics used in cases of infections with multi-drug-resistant strains. The occurrence of such microorganisms may vary depending on the type of wastewater and the season. For many years, the assessment of antibiotic resistance has focused on the study of indicator bacteria. However, there is still a lack of information on the spread of resistant bacteria, not only to well-known and commonly used antimicrobial drugs but also to last-resort antibiotics and those considered as new therapeutic options.

Therefore, **the aim of this project** is to assess the frequency of occurrence of strains from the **ESKAPE** group (*Enterococcus*, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Enterobacter*) resistant to last-resort drugs and new therapeutic options isolated from various types of wastewater, including hospital sewage. Additionally, the project will enable the characterization of the genetic basis of detected resistance in the studied population of strains.

The project will involve sampling from three mechanical-biological wastewater treatment plants with different population equivalent sizes (PE), including raw sewage, preliminary sludge, sludge suitable for agricultural use, and treated wastewater. The research will utilize both traditional methods of microbial cultivation and modern genome analysis techniques. Furthermore, the project plans to detect antibiotics in sewage, which will broaden the dataset and help achieve the goals of project. The following tests will be conducted:

- detection of last-resort antibiotics and new therapeutic options in sewage
- evaluation of the occurrence of ESKAPE pathogens in sewage and sewage sludge
- identification of ESKAPE bacteria with antibiotic resistance mechanisms
- description of the sensitivity of isolated strains to antibiotics, especially last-resort antibiotics and new therapeutic options
- identification of detected resistance genes and mobile genetic elements responsible for resistance to last-resort antibiotics and new therapeutic options.

The fundamental scientific research planned in the project will provide new knowledge about the occurrence of antibiotic-resistant bacteria in sewage with the highest clinical significance and enable the determination of their resistance to last-resort drugs and new therapeutic options. Genome analysis will allow the detection of genes conditioning this resistance. The project's implementation will enable the analysis of consistency between phenotypic and genotypic research results and fill gaps in the current state of knowledge regarding the genetic determinants of bacterial resistance. During the project, it will be particularly important to determine whether the investigated ESKAPE group bacteria spread and persist in sewage at different stages of treatment. Given the undeniable health threat posed by antibiotic resistance and its spread in the environment, the current project aligns with the contemporary trend of research aimed at understanding the coexistence of antibiotic-resistant bacteria in various environments.