Antibiotic resistance is a widespread problem that poses one of the greatest risks to public health around the world. The main cause of antibiotic resistance is the overuse of antibiotics in human and veterinary medicine, and in agriculture. Wastewater reaching wastewater treatment plants (WWTPs) contains antibiotics and/or heavy metals (HMs) that can induce selective pressure. Wastewater from urbanized and industrialized regions is characterized by higher concentrations of these pollutants than wastewater from less industrialized areas. In addition, hospital wastewater entering the sewerage system that conveys municipal wastewater to WWTPs also induces and promotes the spread of antimicrobial resistance determinants of particular clinical significance. Wastewater treatment plants are not equipped with appropriate technologies for eliminating genetic material from treated wastewater. A steady supply of genetic material, including antibiotic resistance genes (ARGs), to aquatic ecosystems turns them into an ideal environment for the acquisition and dissemination of ARGs due to the constant contamination of these ecosystems with antimicrobials of anthropogenic origin.

The aim of the study was to determine the effect of municipal wastewater of various origin on the antibiotic resistance of bacteria in wastewater and river water receiving effluents, and to evaluate the efficacy of photolysis affecting the degradation of antibiotics and antimicrobial resistance determinants in treated municipal wastewater.

The first stage of the study focused on the prevalence of tetracycline resistance genes in samples of wastewater, sludge and river water receiving effluents from two WWTPs differing in the characteristics of influents. The results revealed differences in the prevalence of ARGs depending on the type of influents. In the WWTP located in a tourism region with extensive forest cover, receiving 2% hospital wastewater and 8% industrial wastewater (WM-WWTP), the concentration of the *tet*(M) gene was not reduced during treatment. The *tet*(M) gene was detected on a seasonal basis in samples of activated sludge, treated wastewater, river water and sewage sludge. The research findings confirmed that ARGs cannot be effectively removed during the wastewater treatment process in WWTPs. In the other WWTP, located in an industrialized region and receiving 25% industrial wastewater and 0.4% hospital wastewater (S-WWTP), the analyzed genes were not eliminated during treatment. Seasonal changes in the prevalence of the analyzed genes were not observed. Tetracycline resistance genes were present in all samples of river water. The wastewater treatment process has to be modified to effectively reduce the concentrations of ARGs.

The aim of the second stage of the study was to evaluate the effect of different proportions of industrial and hospital wastewater on the development of antibiotic resistance in municipal wastewater treated in the examined WWTPs (S-WWTP receiving industrial wastewater and WM-WWTP receiving hospital wastewater). Correlations between pollutants such as antibiotics and HMs vs. antimicrobial resistance determinants, and the diversity of genes encoding resistance to beta-lactams, with particular emphasis on carbapenemase genes, during the treatment process were analyzed. Very few significant correlations were noted between the concentrations of antibiotics and ARGs in the examined samples. In turn, HM levels were correlated with ARG concentrations, which confirms the important role of HMs in promoting drug resistance. Significant ($p<0.05$) correlations were also found between the abundance of integrase genes, the abundance of ARGs and the concentrations of HMs. The samples from S-WWTP were characterized by higher HM contamination and a higher number of significant correlations between the analyzed variables than the samples from WM-WWTP. A comparison of wastewater samples from two WWTPs receiving different amounts of hospital wastewater revealed higher diversity of genes encoding resistance to beta-lactams in the samples with a higher proportion of hospital wastewater (58 types) than in those with a lower proportion of hospital wastewater (52 types). In samples from both WWTPs, the dominant types of beta-lactamase genes were *bla*$_{\text{OXA}}$, *bla*$_{\text{GES}}$, *bla*$_{\text{BEL}}$, *bla*$_{\text{CfxA}}$ and *bla*$_{\text{TEM}}$. It is worth noting that *bla*$_{\text{KPC}}$ and *bla*$_{\text{NDM}}$ were detected only in untreated municipal wastewater with a higher proportion of hospital wastewater. The number of correlations between the abundance of beta-lactamases and integrase genes was 94 in wastewater with a higher proportion of hospital wastewater and 41 in wastewater with a lower proportion of hospital wastewater. Therefore, the discharge of effluents from WWTPs to the natural environment may pose a constant threat to human life and health due to the transfer of pollutants such as ARGs, antibiotics and HMs.

The third stage of the study focused on determining the presence of antimicrobial resistance determinants in *Acinetobacter* sp. strains isolated from wastewater samples in each stage of the treatment process, and in river water samples. Subsequently, molecular techniques such as qualitative and quantitative PCR and metagenomic analysis were used to detect the presence of beta-lactam resistance genes (Ambler class B and D), insertion sequence *ISAba1*, class 1, 2 and 3 integron-integrase genes and the taxonomic structure of bacteria. The detection frequency of *Acinetobacte*r sp. and *A. baumannii* was highest in wastewater and river water samples collected in June and September. In the group of resistance genes, *bla*$_{\text{OXA-51}}$ was most prevalent in

Acinetobacter strains. However, metagenomic analysis demonstrated that *bla*$_{\text{OXA-58}}$ was most abundant in the studied samples. The IS*Aba1*/*bla*$_{\text{OXA-51}}$ complex was identified in 13 strains, which confirmed the occurrence of carbapenem-resistance strains in the analyzed samples. The fact that the number of *Acinetobacter* isolates carrying ARGs was not reduced in treated wastewater compared with untreated wastewater suggests that the treatment process was ineffective.

The aim of the fourth stage of the study was to determine the efficacy of removing antibiotics and antimicrobial resistance determinants from treated wastewater by photodegradation. It was found that solar light combined with a photocatalyst (TiO_2) significantly decreased the concentrations of antibiotics and ARGs in wastewater. The tested methods supported the elimination of 52% to 69% of antibacterial agents, and 84% to 90% of the analyzed genes. The incorporation of the above methods to the wastewater treatment system may significantly improve the quality of effluents discharged to the natural environment.

The research findings indicate that the composition of wastewater treated in WWTPs, determined by its origin, significantly affects the development of antibiotic resistance. The number of significant correlations between HMs and antimicrobial resistance determinants was higher in the samples from a WWTP located in a highly industrialized region characterized by high HM contamination than in the samples from a WWTP located in a less industrialized area. It was found that HMs induce antibiotic resistance. In turn, the prevalence of ARGs of particular clinical significance was higher in wastewater samples from a WWTP receiving a higher proportion of hospital wastewater (2%) than in those from a WWTP receiving a lower proportion of hospital wastewater (0.4%). Finally, it was demonstrated that solar-light driven photodegradation used for removing micropollutants from treated wastewater significantly contributes to preventing the spread of antibiotic resistance due to effective antibiotic degradation and a decrease in the abundance of antimicrobial resistance determinants in wastewater.