Wastewater treated by the activated sludge process in municipal wastewater treatment plants (WWTPs) is characterized by very high levels of microbial diversity. In addition to saprotrophic bacteria that participate in the treatment process, wastewater also contains potentially pathogenic and pathogenic bacteria that reach WWTPs with the influent. Antibiotics are also present in wastewater, which promotes the selection of bacteria resistant to antimicrobials. In bacteria, antibiotic resistance mechanisms are encoded by resistance genes which, under favorable conditions, can be transferred to other bacteria. Anaerobic bacteria of the *Bacteroides fragilis* group (BFG) are potential human pathogens that are resistant to many groups of antibiotics.

The aim of this study was to determine the effect of wastewater treatment by the activated sludge process on the antibiotic resistance of BFG bacteria and their spread in the environment.

A total of 319 strains with distinctive features of colonial morphology were isolated from *Bacteroides* Bile Esculin Agar. Of those, 250 demonstrated characteristic resistance to kanamycin, colistin and vancomycin. The PCR test revealed that only 135 of the studied strains harbored the *bfr* gene specific to the BFG. In the MALDI-TOF MS analysis, only 123 isolates were identified to species level or were classified as BFG bacteria. *Parabacteroides distasonis* (51.22% of the strains) and *B. fragilis* (30.01%) were the most prevalent species. In the group of environmental BFG strains, a high percentage of the isolates were resistant to multiple antibiotics, including ciprofloxacin (97.56%), erythromycin (44.71%), ampicillin (35.77%) and tetracycline (32.52%). Antibiotic resistance was noted mostly in the strains isolated from wastewater samples from WWTPs. Genes encoding resistance to tetracyclines (97.78% of all strains that were phenotypically resistant to tetracycline), macrolides, lincosamides and streptogramins (81.97% of all strains that were phenotypically resistant to erythromycin) and chloramphenicol (100% of all strains that were phenotypically resistant to chloramphenicol) were highly prevalent. Genes encoding resistance to β-lactams and fluoroquinolones were less abundant among the analyzed isolates. The highest diversity of antibiotic resistance genes was noted in BFG strains isolated from wastewater samples from WWTPs.

Four dominant bacterial phyla were identified in wastewater samples from 12 WWTPs: *Proteobacteria* (20.4-53.4%), *Firmicutes* (16.1-47.6%), *Actinobacteria* (4.1-40.8%) and *Bacteroidetes* (2.4-15.7%). The class *Bacteroidia* accounted for <1.00% to 8.2% of all bacteria identified in wastewater samples from WWTPs. The proportion of *Bacteroidia*

in samples of untreated and treated wastewater was similar, which indicates that these bacteria are capable of surviving the activated sludge process.

The average concentrations of *bfr* genes and the HF183/BacR287 marker (specific to BFG strains isolated from humans) were similar, ranging from 10^6 copies in 1 ml of untreated wastewater to 10^4 copies in 1 ml of treated wastewater. The analyzed samples, in particular wastewater samples collected from the WWTP in Olsztyn and water samples collected from the Łyna River, were characterized by similar copy numbers of genes specific to the BFG, *E. coli* and *E. faecalis*. Genes encoding resistance to tetracyclines, beta-lactams and MLS antibiotics (macrolides, lincosamides and streptogramins) were characterized by the highest concentrations in wastewater samples collected from 12 WWTPs and water samples collected from the Łyna River. The abundance of genes specific to the BFG was highly correlated with the concentrations of genes characteristic of *E. coli* and *E. faecalis* as well as all antibiotic resistance genes and integrase genes. These results indicate that genes specific to the BFG are highly useful not only in analyses of fecal pollution from anthropogenic sources but also as markers of environmental contamination with genes encoding antibiotic resistance in microorganisms.

All of the analyzed antibiotic resistance genes were highly effectively removed (>93%) in two of the 12 studied WWTPs. These plants deployed systems for highly efficient removal of biogenic substances. The sludge was young, and the hydraulic retention time of wastewater in the bioreactor was relatively short. In samples of treated wastewater collected from a WWTP with the oldest sludge, the abundance of nearly all analyzed genes increased after treatment.

The analysis of the studied WWTPs' influence on receptacles of treated wastewater demonstrated a significant increase in the copy numbers of genes specific to the BFG, *E. coli,* and 7 out 11 antibiotic resistance genes in samples of river water collected downstream from the wastewater discharge point. These findings indicate that WWTPs contribute to the spread of antibiotic resistance genes in rivers.

The present study was conducted as part of doctoral research, and it contributes new knowledge on the impact of activated sludge WWTPs on the prevalence, antibiotic resistance and transmission of BFG bacteria to the environment. This study also demonstrated that genes specific to the BFG can be used as markers of anthropogenic pollution in aquatic environments.