SUMMARY

Antibiotic resistance is a critical global health problem, driven by the use of antibiotics in health care and agriculture, which leads to environmental contamination with antibiotic residues and resistant bacteria. These contaminants, even at low levels, can drive resistance through selective pressure and horizontal gene transfer, posing risks to human, animal and environmental health systems, as the framework of “One Health” emphasizes. Aquatic environments are particularly vulnerable, receiving contaminants from wastewater, agriculture, aquaculture and industrial activities, yet research on antibiotic resistance in these settings is limited, especially beyond wastewater treatment plant discharges. To address gaps in surveillance, novel UHPLC-MS/MS methods were developed for a comprehensive analysis of antibiotic residues in water and sediments. By integrating the study of antibiotic residues and resistance, this research contributes to understanding the environmental transmission of resistance and informs policy development. Current environmental monitoring frameworks remain inadequate, highlighting the need for more comprehensive approaches to manage this growing threat.

This project investigated two major pollution hotspots. First, waterways located partly in regions with a lot of livestock agriculture in Flanders (Belgium), were studied. These waterways were chosen due to their high susceptibility to contamination from manure application. Surface and groundwater samples were collected before and after fertilization to assess antibiotic residues and resistance, focusing on *E. coli* as a fecal indicator with additional interest in the production of extended-spectrum β-lactamases. Secondly, two Belgian seaports, Nieuwpoort and Oostende, were investigated for antibiotic residues and resistance in water and sediments, analyzing organisms such as marine indicators *Shewanella* and *Vibrio* and ESBL-producing *E. coli*.

In freshwater, antibiotic residues (ABRs) were detected in 78% of the surface water samples, with 25 different residues identified, most frequently lincomycin and sulfonamides. Concentrations ranged from 0.01 µg/L to 8.83 µg/L. *E. coli* were present in 94-98% of the surface water samples, while suspected extended-spectrum β-lactamase (ESBL)-producing E. coli were detected in 26%, averaging 1% of total *E. coli*. Groundwater samples showed much lower bacterial counts and no ESBL-producing *E. coli*. In surface water, resistance to sulfamethoxazole in *E. coli* increased from 20% to 48% after fertilization. ESBL-producing *E. coli* showed higher resistance to non-β-lactam antibiotics compared to generic *E. coli*. The Predicted No Effect Concentrations (PNECs) for resistance selection were exceeded five times in freshwater (lincomycin and sulfadiazine), highlighting the risk of resistance selection. The persistent presence of sulfonamides and rising sulfamethoxazole resistance indicate the impact of veterinary antibiotic use on aquatic systems. In the marine environment, sediments contained higher concentrations of ABRs, up to 25 µg/kg, consisting mainly of quinolones and macrolides, while sulfonamides dominated in water samples. This co-occurred with higher resistance rates for sediments which showed higher resistance rates for quinolones among suspected ESBL-producing *E. coli*. *Shewanella* and *Vibrio* species were widespread. *Shewanella* showed resistance to colistin and one isolate was resistant to meropenem. Resistance in *Vibrio* was similar, but further testing is needed to draw more definitive conclusions. The work of this project demonstrates a pressing need for comprehensive environmental surveillance of antibiotics and resistance. Ultimately, these insights can inform policy frameworks to mitigate antibiotic resistance in environmental contexts.

Keywords: Antibiotic resistance, antibiotic residues, One Health, water, *E. coli*