Title: Potential Role of Treated Wastewater and Heavy Rainfall Events on Environmental Spreading of AMR into the Lambro River (Lombardy Region, Italy)

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Background: Antibiotic-resistance indicators (ARB, ARGs, and MGEs) are frequently detected in environmental matrices, including Wastewater Treatment Plant (WWTPs) effluents. Wastewater Bypass could also contribute to their dissemination, especially during stormwater events. This project aimed to assess the potential impact of heavy rainfall on the release of ARGs and ARB into the receiving river.

Method: Water samples were collected along the Lambro River (Northern Italy), at five sampling points, in the absence and in the presence of high-intensity rainfall events. ARB were quantified using culture-based methods. DNA was then extracted for the quantification of *16S rRNA*, *intI1*, and seven ARGs. Bacterial community composition was investigated via *16S rRNA* gene Amplicon Sequencing on MiSeq – Illumina platform.

Results: In absence of rainfall, several ARGs showed significantly higher concentrations downstream of the WWTP compared to upstream, without a corresponding increase in culturable ARB. During rainfall events, both ARGs and ARB significantly increased along all the river sites, suggesting a potential contribution of wastewater bypass, stormwater runoff, and potentially additional upstream sewer overflows. PCA analysis revealed that the riverine bacterial communities collected during rainfall clustered closely with wastewater influent samples, highlighting a strong wastewater-related signature in the river during high-intensity rainfall conditions.

Conclusions: WWTP effluents may contribute to the environmental dissemination of ARGs in absence of extreme weather events, while rainfall acts as a major driver of widespread AMR dissemination, highlighting the anthropogenic pressure on the Lambro River and the importance of considering hydrological conditions when assessing environmental AMR dynamics.