

**Title:**

Ripples of Resistance: Unveiling AMR Dynamics Along Switzerland's Aare River

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**Abstract (300 words maximum): :**

The global spread of antimicrobial resistance (AMR) is a severe public health threat, driven by widespread antibiotic use and the environmental release of antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs). Large rivers, heavily influenced by wastewater (WW) discharge and serving as essential resources to large populations, can be key contributors to AMR dissemination. However, the dynamics and drivers of AMR along entire river systems remain poorly understood.

This study investigates the impact of WW inputs from six wastewater treatment plants (WWTPs) and tracks ARG progression along 288 km of the Aare, Switzerland's longest river. Combining flow cytometry, quantitative PCR of indicator genes, and 16S rRNA amplicon sequencing, the study provides a high-resolution analysis of ARG dynamics from the river's source to its confluence with the Rhine.

Fifteen ARGs linked to eight clinically relevant antibiotic classes, along with the integron integrase gene (*intI1*) and the beta-glucuronidase gene for *E. coli* detection, were quantified and linked with environmental and nutrient parameters. WWTP effluent exhibited up to 3'160 times higher ARG levels than the receiving river, increasing concentrations downstream of major discharge points by a factor of 1.04 to 140. ARG abundance progressively increased along the river, driven by the WW discharge of Interlaken, Bern, and Aarau and sustained by tributaries such as the Reuss and Limmat. Hotspots were identified at upper Wohlensee (downstream of Bern) and near Brugg, close to the estuary. Interestingly, a "reset" in ARG levels was consistently observed where the Aare River passes various lakes. This effect may be attributed to particle sedimentation and the exchange of the microbial community during the considerable residence time of water in the lakes.

This study establishes the first baseline for ARG prevalence in the Aare River, offering valuable insights into AMR dynamics and informing future monitoring and mitigation strategies.