

## **Resistance gene profiles in rock glacier-influenced alpine catchments: links to metal gradients and hydrogeochemistry**

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Antimicrobial resistance represents a global health threat, yet its environmental drivers remain poorly understood. Heavy metals may contribute to the selection and maintenance of antimicrobial resistance genes (ARGs) alongside metal resistance genes (MRGs). Rock glaciers represent long-term water reservoirs and, in our study area, release trace metals to downslope waters. We investigated taxonomic and MRG/ARG profiles in substrates from six rock glaciers with different degrees of activity and permafrost content and nearby reference springs in the Eastern Alps across two seasons, assessing associations between MRGs and ARGs and the influence of geochemical and physical conditions. Shotgun metagenomic and geochemical data were analysed. Taxonomic composition indicated a conserved core microbiome, constant within the same location, with no seasonal variation. ARG and MRG profiles differed more by valley than by site type, with a smaller seasonal effect. Ordination analyses identified Cd, Ce, and temperature as significant correlates of ARG and MRG profiles; notably, cadmium was significantly and positively associated with the corresponding resistance genes. Across all samples, ARG abundance was positively correlated with MRG abundance (Spearman  $\rho = 0.894$ ,  $p < 0.001$ ). PLS-PM (GoF = 0.609) supported a dominant pathway in which trace-metal gradients were positively associated with MRGs, and MRGs strongly predicted ARGs. Overall, MRGs strongly co-varied with ARGs across samples. While both resistomes were shaped by geochemical gradients and valley-specific characteristics, MRGs were more closely associated with trace elements, suggesting that trace-metal dynamics should be considered in future monitoring and management of alpine headwater systems, particularly under ongoing climate change.

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