## Antibiotic resistome dynamics in agricultural river systems: Elucidating transmission mechanisms and associated risk to water security

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Usage of antibiotics in agriculture has increased dramatically recently, significantly raising the influx of antibiotic resistance genes (ARGs) into river systems through organic manure runoff, seriously threatening water security. However, the dynamics, transmission mechanisms, and potential water security risk of ARGs, as well as their response to land use spatial scale and seasonal variations in agricultural river systems remain unclear. To address these challenges, this work employed metagenomic technique to systematically evaluate the pollution and dissemination of ARGs in overlying water and sediment within a typical agricultural catchment in China. The results demonstrated significant differences between overlying water and sediment ARGs. Overlying water dominated by multidrug ARGs exhibited higher diversity, whereas sediment predominantly containing sulfonamide ARGs had higher abundance. The dynamics of ARGs in overlying water were more responsive to seasonal variations compared to sediment due to greater changes in hydrodynamics and nutrient conditions. The profiles of ARGs in overlying water were largely regulated by microbiota, whereas mobile genetic elements (MGEs) were the main forces driving the dissemination of ARGs in sediment. The variation in dissemination mechanisms led to different resistance risks, with sediment presenting a higher resistance risk than overlying water. Furthermore, Mantel test was applied to discover the impact of land use spatial scale and composition on the transmission of ARGs in river systems. The findings showed that cultivated land within 5 km of the riverbank was the key influencing factor. Cultivated land exacerbated ARGs spread by increasing MGEs abundance and nutrient concentrations, resulting in the abundance of ARGs in high-cultivated sites being twice that in low-cultivated sites, and raising the regional water security risk, with a more pronounced effect in sediment. These findings contribute to a better understanding of ARGs dissemination in agricultural watersheds, providing a basis for implementing effective resistance control measures and ensuring water security.

