Spatial ecology of a wastewater network defines the antibiotic resistance genes in downstream receiving waters

Wastewater treatment plants (WWTPs) are an effective barrier in the protection of human and environment health around the world, although WWTPs also are suggested to be selectors and/or reservoirs of antibiotic resistance genes (ARGs) before entering the environment. The dogma about WWTPs as "ARG selectors" presumes that biotreatment compartments (e.g., activated sludge; AS) are single densely populated ecosystems with elevated horizontal gene transfer. However, recent work has suggested WWTP biotreatment compartments may be different than previously believed relative to antibiotic resistance (AR) fate, and other process factors, such as bacterial separation and specific waste sources, may be key to ARGs released to the environment. Here we combined 16S rRNA metagenomic sequencing and high-throughput qPCR to characterise microbial communities and ARGs across a wastewater network in Spain that includes both community (i.e., non-clinical urban) and hospital sources. Contrary to expectations, ARGs found in downstream receiving waters were not dominated by AS biosolids (RAS), but more resembled raw wastewater sources. In fact, ARGs and microbial communities in liquid-phase WWTP effluents and RAS were significantly different (Bray–Curtis dissimilarity index = 0.66 ± 0.11), with a consequential fraction of influent ARGs and organisms passing directly through the WWTP with limited association with RAS. Instead, ARGs and organisms in the RAS may be more defined by biosolids separation and biophysical traits, such as flocculation, rather than AR carriage. This explains why RAS has significantly lower ARG richness (47 ± 4 ARGs) than liquid-phase effluents (104 ± 5 ARGs), and downstream water column (135 ± 4 ARGs) and river sediments (120 ± 5 ARGs) (Tukey's test, p < 0.001). These data suggest RAS and liquid-phase WWTP effluents may reflect two parallel ecosystems with potentially limited ARG exchange. As such, ARG mitigation in WWTPs should more focus on removing bacterial hosts from the liquid phase, AR source reduction, and possibly disinfection to reduce ARG releases to the environment.