Unravelling antimicrobial resistance spread within populations trough integrated metagenomic and culturomic analyses of wastewaters.

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Antimicrobial resistance (AMR), occurring when pathogens evolve to withstand antimicrobial drugs, is making infections more difficult to treat and can be regarded as a "silent pandemic" that may cause up to 10 million deaths annually by 2050. In this scenario, wastewater-based epidemiology (WBE) may contribute to the surveillance of AMR diffusion and evolution. We implemented WBE to investigate AMR in specific settings, such as those characterized by high antibiotic usage (e.g. hospitals) and those representative of a young and healthy population (e.g. universities), in comparison with the whole community, by analysing different sewage sources from the same urban environment. ESBL-producing *Escherichia coli* prevalence was similar regardless the sewage source, coherently with the widespread use of cephalosporins. On the contrary, vancomycin resistant *Enterococci* (VRE), among major causes of healthcare-associated infections, were higher in wastewater from the hospital, where this antibiotic is manly used. Whole genome sequencing and phenotypic characterization indicated the occurrence of peculiar resistance features of hospital-derived strains. Quantification by qPCR/dPCR of selected antimicrobial resistant genes (ARGs), together with metagenomic-based high-throughput profiling of ARGs, showed population-specific resistance patterns, with a higher abundance of ARGs related to clinically relevant resistances in sewages representing the hospitalized population. Taken together these results indicate how the nosocomial environment, characterized by a large use of antibiotics, may represent a hot-spot for AMR. The application of WBE to specific settings provides high-resolution clues on the dimension of AMR within specific sub-populations, contributing to the definition of public-health interventions to combat antimicrobial resistance.