

A flowing database: Harnessing sewage-based surveillance for antimicrobial resistance

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Antimicrobial resistance poses a major challenge to modern medicine and jeopardizes the ability to maintain a robust global public health response against persistent infectious disease threats. A 2024 *Lancet* series on antimicrobial resistance estimated that bacterial antimicrobial resistance was associated with 4.71 million deaths globally in 2021, including 1.14 million deaths directly attributable to resistant infections. If not actively addressed, antimicrobial resistance might result in an annual reduction of the global gross domestic product by \$3.4 trillion and push an additional 24 million individuals into extreme poverty within the next decade. Antibiotic resistance genes (ARGs) are the key culprits behind antimicrobial resistance, widely disseminating among bacterial populations, particularly in medical, agricultural, and veterinary sectors. Furthermore, anthropogenic activities, such as wastewater discharge, trade, tourism, and chemical contamination, have reshaped microbial biogeography, creating an unforeseen global platform for ARG mobilization. This exacerbates the existing antimicrobial resistance crisis and enhances risks to global public health and environmental stability.¹

Conventional wastewater surveillance systems typically involve monitoring community drug use; tracking the consumption of substances such as nicotine, caffeine, and alcohol; and measuring exposure to environmental contaminants. Mounting evidence indicates that wastewater may serve as an optimal environment for epidemiological surveillance. Sewage-based surveillance is an emerging field that systematically examines the presence of infection biomarkers, such as pathogen/virus DNA or RNA shed by infected individuals, in both treated and untreated municipal wastewater using molecular and genomic tools. Hong Kong's city-wide sewage-based surveillance program effectively guided COVID-19 responses by tracking wastewater virus levels in real time. The global spread of the SARS-CoV-2 infection has propelled sewage-based epidemiology as a potent surveillance tool in public health, offering early detection of infectious disease outbreaks within community populations sharing a common sewer system or drainage area²; yet, surveillance of ARGs is frequently still in its fledgling stages.

Current ARG surveillance relies on the passive reporting of laboratory results for specific pathogens isolated from humans or contaminated environments, incurring substantial time and economic costs. In contrast, sewage-based surveillance aggregates data from various sewer sheds to reflect the collective health status of a community (Figure 1). A strategically designed and comprehensive sewage-based surveillance system has the potential to establish a genomic framework for comparing baseline levels of antimicrobial resistance within a community, identifying concerning trends for prompt intervention, and predicting major developments at local, regional, and national scales. Compared with metagenomics and shotgun sequencing, the surveillance necessitates the use of highly sensitive techniques capable of comprehensively assessing ARG prevalence and abundance, even at low levels undetectable by current metagenomics methodologies. In this context, digital polymerase chain reaction (PCR) and highly parallel quantitative PCR offer high sensitivity and specificity, enabling accurate quantification of nucleic acids even at low levels, and both can be applied in various fields like genomic, clinical diagnosis, etc. The superior accuracy of advanced PCR methods justifies their cost for surveillance, with improving affordability and significant long-term public health returns offsetting initial investments. Though ARGs are environmentally ubiquitous,

sewage monitoring of low-level ARGs provides vital public health intelligence by tracking community resistance patterns and emerging threats, enabling proactive interventions. Digital PCR excels in absolute quantification without the need for standard curves and offers high sensitivity for detecting rare targets; however, it has higher costs and lower throughput compared to a highly parallel qPCR. In contrast, highly parallel qPCR analyzes up to 384 primer sets simultaneously in nanoliter volumes, reducing per-sample costs and labor for large-scale studies. Pärnänen et al. conducted a groundbreaking trans-European survey on antimicrobial resistance using the highly parallel qPCR, revealing a strong correlation between environmental and clinical antimicrobial resistance through the analysis of ARG profiles in urban wastewater.³

Sewage-based epidemiology is increasingly used for antimicrobial resistance surveillance, offering notable advantages in accessing extensive information regarding community health status, while serving as an early warning indicator for resistance threats. In contrast to isolate-based testing from hospital settings, sewage-based surveillance aggregates data from a broad non-hospital population, capturing the regional resistance landscape through pooled samples. Currently, no standardized guidelines exist for interpreting antimicrobial resistance surveillance data from sewage samples. Establishing a standard for the use and interpretation of antimicrobial resistance data is of utmost significance, as it ensures consistent, reliable, and meaningful analysis. Unlike clinical testing, which relies on data patterns from a subset of consenting individuals, sewage-based surveillance would provide unbiased testing by gathering pooled data from all individuals within a specific area. This streamlined approach enables decision-makers to implement timely interventions, potentially averting outbreaks of antimicrobial resistance. Additionally, it allows for the monitoring of intervention effects, irrespective of the capacity to isolate and identify bacterial pathogens; facilitating the identification of high-risk populations; and forecasting evolutionary trends by leveraging robust models and extensive ARG prevalence data. It is also non-invasive, meaning that it avoids the direct sampling of individuals while allowing for monitoring of temporal trends and adaptation processes to disease outbreaks.

Integrating statistical models encompassing various environmental and hydro-meteorological factors enhances our understanding of the dynamics and fates of ARGs in wastewater. This comprehensive approach offers insights into the spatial and temporal distribution of antimicrobial resistance, aiding in risk assessment and management strategies. Furthermore, sewage-based surveillance presents a cost-effective environmental monitoring solution through the concept of "one sample, many analyses". By simultaneously examining environmental samples for antibiotic-resistant bacteria and ARGs and conducting diverse molecular and chemical analyses, water samples can serve as sentinels for environmental health assessment. Cost estimates for sewage-based surveillance indicate less than 0.1% of total costs compared to clinical testing.⁴ It suggests that nationwide implementation of sewage-based surveillance as a complement to clinical measures could yield significant cost savings without compromising surveillance accuracy, potentially leading to substantial financial benefits amounting to millions or even billions of dollars.

Sewage surveillance for antimicrobial resistance offers significant advantages as an early warning system, yet it faces inherent limitations that could impede its long-term development. While sewage-based surveillance can

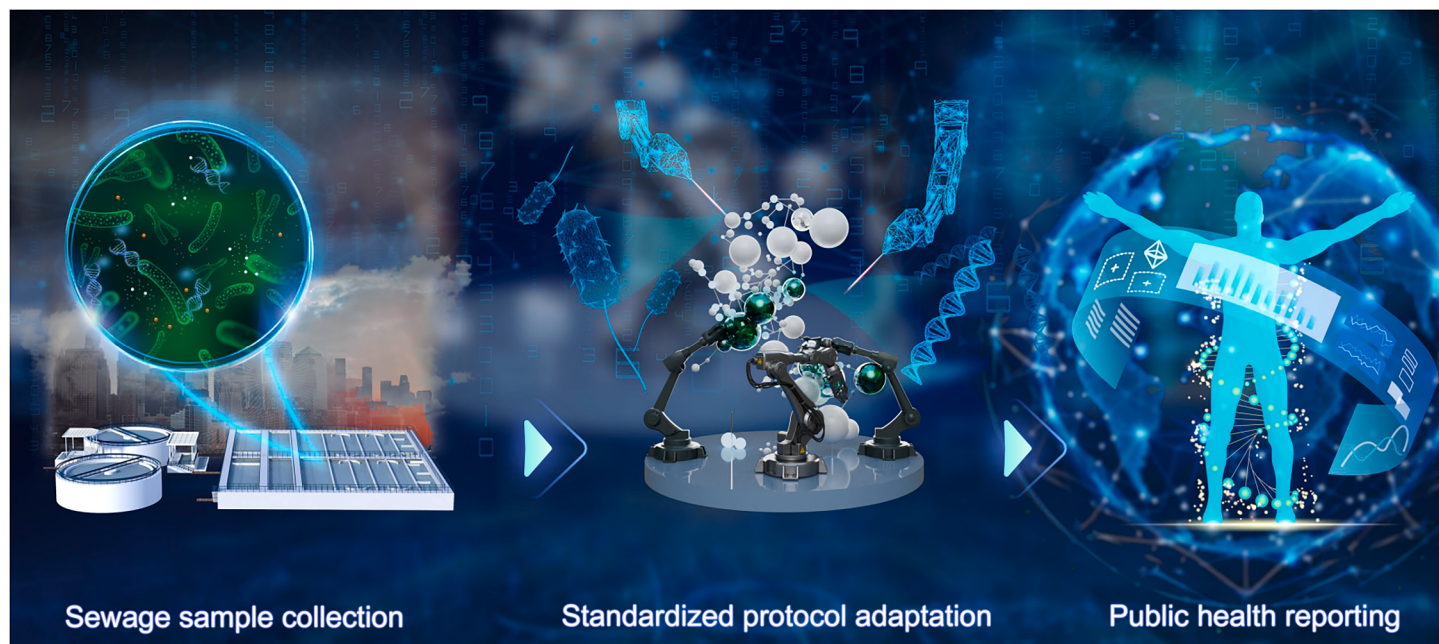


Figure 1. Sewage-based surveillance as a powerful public health tool

quantify ARGs using high-throughput qPCR, even at low concentrations, the detected ARGs represent the overall resistance prevalence within the microbial community of wastewater and cannot be attributed to specific bacterial species. High variations of bacterial diversity among different wastewater surveillance systems thus complicate generalized conclusions. Complex matrices within the wastewater, as well as dilution effects by rainfall and incoming tributaries, pose additional challenges to analytical detection limits. Additionally, there are challenges in assessing the risk level of potential ARGs, particularly since adjacent data from individuals might be lacking. Many intrinsic genes with high abundances existed before the clinical use of antibiotics, forming part of the natural resistome and encoding physiological functions without conferring resistance, posing a minimal threat to human health. To better assess the risk of ARGs to human health, criteria such as human accessibility, pathogenicity, and gene mobility should be considered. This could involve categorizing ARGs into four risk levels: significant risk (human-associated, pathogenic, and mobile ARGs), high risk (human-associated, pathogenic, and non-mobile ARGs), general risk (human-associated and non-pathogenic ARGs), and low risk (human-unrelated ARGs).⁵ Quantitatively evaluating the potential risk level of ARG pollution can help identify human-associated and pathogenic ARGs, enabling targeted remediation strategies to control resistance in wastewater. Besides, mounting evidence connects environmental origins of antifungal resistance to clinical outcomes, underscoring the need for balanced research on environmental microbiomes, human fungal pathogens, and antimicrobial resistance to combat this public health threat.⁶ Beyond methodological debates, there is an urgent need for a standardized framework. ARG sewage surveillance should cover aspects such as sampling site representativeness, frequency and timing, sample pretreatment for DNA enrichment, extraction and purification, high-throughput qPCR detection, and data interpretation and reporting. This would facilitate the acquisition of high-quality, reliable, and comparable results, laying the groundwork for global data sharing. Wastewater, often referred to as the “dirty blood of the city”, can rapidly transfer clinically relevant ARGs into human-impacted environments through waterborne-aerosolized or waterborne-foodborne pathways. To effectively and timely manage antimicrobial resistance and minimize life-threatening infections, efforts to streamline the process from sampling, DNA extraction, quantification, and data interpretation to response actions and realize “real-time” sewage-based surveillance deserve significant support. Finally, while sewage-based surveillance provides population-wide information that complements other epidemiological systems, it cannot independently elucidate pathogenicity or underlying inducing factors. Future challenges might arise from novel ARGs and complex interactions between mobile genetic elements, multiple other ARGs, and

pollutant mixtures, promoting multidrug resistances. Yet, integrating sewage surveillance with other epidemiological methodologies to address initial resistance from established disciplines is a promising and significant endeavor.

Rising populations and global warming will intensify human-pathogen interactions and drive pathogen evolution, necessitating ongoing technological innovation to maintain effective surveillance of emerging threats. Urban wastewater is akin to a treasure trove, rich with a diverse of bio-chemical substances that mirror various facets of human metabolism, including lifestyle, diet, health status, and exposure to stressors. Sewage-based surveillance emerges as a valuable and innovative approach, enabling the collection of epidemiological information through urban wastewater monitor, swiftly and effectively tracing shifts in the health status of a population. Consequently, this sentinel system should be expanded to encompass the monitoring of additional pathogens and their associated ARGs with the aim of enhancing public health preparedness.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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