

Sewage surveillance of antibiotic resistance holds both opportunities and challenges

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A proposed European Union-directive requests that member states monitor antibiotic resistance at all sewage treatment plants serving >100,000 people. Sewage surveillance could provide information on the resistance situation in the underlying population and on environmental transmission risks. There are opportunities to make such surveillance data more informative and actionable, but there are also challenges.

The environment has several roles in the global antibiotic resistance crisis. The diverse ecological niches in the environment provide a vast reservoir for new antibiotic resistance genes that over time accumulate in pathogens. Polluted waters and soils are important transmission routes for several pathogens, including antibiotic-resistant enteric bacteria. From a more backward-looking perspective, studying resistance in the environment can provide a reflection of the resistance situation in the regional human (or domestic animal) population. Environmental surveillance of antibiotic resistance could therefore serve multiple purposes^{1,2}.

Although sewage surveillance for poliovirus has been in place for decades, the COVID-19 pandemic gave this approach a new lease of life as a resource-efficient tool for monitoring the spread and evolution of SARS-CoV-2. The view on sewage as a resource for health care is likely to have had a role for the European Commission in their proposed revision of the Urban Waste Water Treatment Directive (UWWTD)³. The proposal emphasizes the role of sewage surveillance for preventive or early warning purposes, exemplified by the detection of specific viruses as a signal of the emergence of epidemics or pandemics. It also recognizes emissions from sewage treatment plants as a major transmission pathway for antimicrobial agents and resistant bacteria. However, the specific motivation for sewage surveillance of antimicrobial resistance (AMR) is primarily to increase the knowledge on main sources of antimicrobial resistance to the environment. We think that it would be a lost opportunity if such a surveillance effort would not aim to both address environmental transmission risks from sewage treatment plants and inform about the resistance situation in the primary source for many of these bacteria – the regional human population. The draft UWWTD requests sampling of both influents (untreated urban sewage) and treated effluent. This would perfectly match such dual objectives, as carriage in the population is best reflected by samples taken as close to

the source as possible (influent), whereas environmental transmission risks are determined by what is released (effluent).

In parallel to the UWWTD, the US Centers for Disease Control and Prevention (CDC) is planning or already building capacity for AMR surveillance in wastewater treatment plants and health-care institutions across the USA. In some contrast to the UWWTD draft, the CDC initiative includes the explicit objective of reflecting AMR in the contributing human population, particularly silent outbreak detection⁴. Sewage surveillance has benefits compared with, and complements, traditional clinical surveillance as it is exceptionally resource efficient; that is, one sample can represent bacteria from hundreds of thousands of people. For this reason, it also enables early outbreak detection of rare or even novel forms of resistance using, for example, selective culture techniques, targeted PCR, tailored functional metagenomics or shotgun metagenomics combined with modelling^{5,6}. Furthermore, it comes without the (often) strong bias resulting from different practices for collecting samples from patients in different regions, thus making data more directly comparable. However, it differs, for good and bad, in that sewage bacteria come from both the healthy and sick part of the population, even if taken from hospital sewers. Depending on the endpoint measured, the large contribution of bacteria in sewage that cannot be attributed to the human microbiota⁷ could also make interpretations challenging⁵.

Although some regions of the world, including Europe and the USA, have reasonably well-developed clinical surveillance systems, many countries lack systematic generation and collection of resistance data. The Global Antimicrobial Resistance and Use Surveillance System (GLASS) initiative by the World Health Organization is an important step in creating a foundation for harmonized data collection, but lack of resources will probably still be a limiting factor for a considerable time. Without surveillance data, physicians rely on their own experience of which antibiotics do or do not work. In such situations, a resource-efficient sewage surveillance system, based on isolates, would have the potential to directly inform empirical treatment and thus save lives. Such use, however, requires extensive benchmarking against clinical surveillance data⁸. Generating quantitative sewage surveillance data on a broad scale in Europe and the USA could therefore contribute to such an evaluation. This could, in turn, facilitate implementation of quantitative sewage surveillance as one additional means for combating bacterial infections and resistance in low-resource regions. Given how easily resistance spreads across the globe, it matters for every region how resistance is managed in each part of the world.

Before designing any surveillance action, the knowledge gaps that need to be filled (and why) should be specified, the type (or types) of data that could be collected and that would efficiently close those gaps should be identified, and feasible actions as a response to the

new knowledge should be envisioned⁹. Surveillance data that do not fill an important knowledge gap or are not actionable are not very useful. It should be said that directing further research is a valid action, because it may request more than surveillance data to direct effective mitigations. For example, even if we know the exact level of discharged resistant bacteria from urban sewage, assessing risk of transmission to humans or animals would still require more knowledge on exposure levels and to what extent the exposure leads to colonization or disease. Depending on the objective (or objectives) of sewage surveillance, different endpoints (such as antibiotic residues, resistance genes or (absolute or relative) abundances of certain resistant bacteria) carry vastly different information value¹. We therefore recommend a thorough exercise defining objectives, specifying knowledge gaps, selecting informative endpoints and envisioning potential actions to get the most value out of the effort. In contrast to viral pandemics, the silent pandemic of antibiotic resistance is slow, although it is continuously escalating in severity. Hence, sampling less frequently than twice a year, as indicated in the UWWTD, might be sufficient and instead allow analysis of a wider panel of endpoints that address the different objectives within a limited budget.

The Global Sewage Project has pioneered the field using metagenomic analyses, demonstrating feasibility in more than 100 countries¹⁰. Lessons learned include that centralized sample processing, sequencing and bioinformatic analyses strongly reduce total workload and increase comparability. Collected data on resistance in sewage can be either gene-based from communities (metagenomes) or phenotypic (cultivation-based). We have recently elaborated on the pros and cons with these strategies with regard to quantitative assessments of the regional resistance situation⁶. There are similar pros and cons with regard to transmission risks; analyses of resistance genes (for example, from PCR or short-read metagenomics) in environmental matrices without reliable host information are challenging to translate into any quantifiable transmission risk. In large parts, this is because most resistance genes occur in a range of different contexts and hosts, many of which do not pose any direct clinical threat (without involving horizontal gene transfer). Long-read sequencing may overcome some, but not all, of these limitations. Translation into transmission risks is considerably more straightforward from cultivation-based data, although such data collection may come with somewhat higher costs and is restricted to the cultured organism. Given the distinct strengths and weaknesses, we would currently recommend a combination of cultivation and cultivation-independent approaches. Highly specific methods for selective isolation of species other than *Escherichia coli* in complex

sewage currently limit some of the expansions of a cultivation-based approach.

The recent initiative by the European Commission to include surveillance of antibiotic resistance is good and could prove to be important for addressing a critical health issue. Still, without paying careful attention to both possibilities and challenges, opportunities may be lost and/or many resources could be spent on collecting data that contributes little to actions.

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Published online: 5 December 2022

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Competing interests

The authors declare no competing interests.