Wastewater and Environmental Surveillance Summary for Antimicrobial Resistance

Pilot version,1 December 2025

This document provides information on wastewater and environmental surveillance (WES) for antimicrobial resistance (AMR), including AMR pathogens and antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs). It should be used together with the accompanying *WES Guidance for one or more pathogens*, which includes general and cross-cutting information (available here).

WES for AMR at a glance

- AMR refers to the ability of microorganisms to show reduced sensitivity to antimicrobial agents, and is of high and growing global public health significance: a top ten global health threat.
- WES for AMR is technically and operationally feasible. Some pilot studies have shown good correlations between clinical and wastewater prevalence and proportions of AMR pathogens.
- WES for AMR has the potential to be actionable and acceptable but has not been in routine use. Pilot and research studies are rapidly increasing, and set to grow significantly, particularly in the European Union following the obligation to test for AMR in effluent of large wastewater treatment works, and its consideration for use in the US NWWS.
- Integration of AMR phenotype and ARG targets with other targets within one or more WES workflows has been demonstrated in pilot studies but not been demonstrated routinely.

Table 1: At a glance assessment of key WES criteria for AMR (sewered and non-sewered)[a,b]

Setting	Categorical Assessment (CA) / Strength of Evidence (SoE)	Public Health Significance	Actionability / Relative value	Technical Feasibility	Operational Feasibility	Acceptability	Optimisation	
							Integrated disease response	Multitarget WES
Sewered	CA	High	Intermediate	High	Intermediate	High	High	Low
	SoE	Strong	Inadequate evidence	Moderate	Inadequate evidence	Moderate	Moderate	Moderate
Non-sewered	CA	not separated by sewered category	Low	High	Low	High	Low	Low
	SoE		Inadequate evidence	Inadequate evidence	Inadequate evidence	Moderate	Inadequate evidence	Inadequate evidence

Key:

1. Categorical Assessment (CA) of criteria

Category	Code	Description
High		Criteria is evaluated as met at the highest level
Intermediate		Criteria is evaluated as met at an intermediate level (it may be that not all sub-components of the criteria are met)
Low		Criteria is evaluated as low
Not-supported		Criteria is evaluated as not supported
Not applicable		Criteria is not applicable OR cannot assessed due to inadequate evidence

2. Strength of evidence (SOE)

Evidence level	Code	Description
Strong		High quality consistent evidence, including from multiple relevant studies/settings, at scale, over a prolonged period, with evidence from program settings, not only from research studies or short projects.
Moderate		Relevant evidence is available but does not meet criteria for 'Strong' classification.[c]
Inadequate evidence		Evidence is inadequate and further study/evaluation is needed

[a] Further description of the criteria used to assess the applicability of WES for a specific pathogen, as well as the methods used to evaluate them, is included in *WES Guidance for one or more pathogens*. The assessment in Table 1 provides a snapshot at the global level, but country level assessment may differ.

[b] Sewered settings refers to closed reticulated sewage systems. Non-sewered settings refers to the diverse settings which are not 'sewered', including open drains and community sampling points. Individual small septic tanks at residential or building level are not viable to sample individually and are not considered here separately. Most WES evidence to date is reported from reticulated sewered settings, often from high-income settings. Yet much of the global population is on heterogenous non-sewered systems and this has implications for assessment of various WES categories.

[c.] Evidence classified as 'Moderate' meets one or more of the following criteria: not from numerous settings, for a short period, without program-level evidence, and/or where findings are not consistent or of high quality.

Summary

Key features of WES for this target suite

- AMR is a globally significant property of circulating microorganisms (primarily bacteria, but also fungi, parasites, and viruses) whereby those microorganisms become less sensitive to one or more antimicrobials, making pathogenic microorganisms harder to treat.
- AMR pathogens and ARG are widely dispersed, and more so in settings that use more antimicrobials (e.g. medicine and agriculture).
- Whilst AMR is not directly vaccine-preventable, vaccines may be effective against some AMR pathogens, and can be given to at-risk groups and in response to outbreaks if warranted.
- Global, regional and national agencies have monitoring and management programs, based on clinical testing which WES could support.
- WES for AMR should be undertaken in the context of broader AMR surveillance to support those existing efforts, and within the One Health context due to animal and environmental reservoirs.
- There are decades of experience with sampling and testing of wastewater, and to a lesser extent environmental waters, for AMR pathogens and ARGs.
- Most studies are pilot or 'proof of concept', with no standard methods having emerged.
- Many studies showed good correlations between wastewater and clinical results, particularly in hospital wastewater. Others have poorer correlations, possibly due to confounding factors, such as animal inputs, infections in humans that are not diagnosed, or microbial ecological drivers of AMR. Environmental waters in non-sewered areas are poorly studied.
- Results are not currently being utilised to inform public health actions, with routine WES AMR programs not established, and triggers for action not having been developed.
- Most of the 'proof of concept' studies are related to just a small number of AMR pathogens and ARGs, albeit these include most of the highest priority pathogen-antimicrobial combinations.
- There are no examples of routine WES with health-impactful actions in multiple independent settings to support a program or provide a supporting benefit-cost analysis.
- The target sheet does not present routine WES or standard methods, use cases, or approaches. Rather, it sets out prioritization processes to determine the pathogen-antimicrobial combinations to target, pilot studies to help prove methods and their sensitivity and specificity, and develop triggers for public health action including antimicrobial stewardship.
- Key questions to test with future research are:
    - What pathogens-antimicrobial combinations are highest priorities for each setting.
    - What are the preferred sampling, analysis and bioinformatics workflows and how sensitive and specific are they?
    - What are the demonstrated health-impactful use cases to respond to WES evidence?

Key considerations for WES for AMR and ARGs

Consideration	Suggestion
Pathogen-antimicrobial combinations to include in the AMR WES program	*Select a manageable number (typically three to six) based on local priorities:* • *Base this selection on high disease burden pathogen-antimicrobial combinations for which clinical data is inadequate, e.g. due to currently low prevalence or gaps in existing surveillance.* • *Refer to Table 2 for a global perspective on high priority pathogen- AMR combinations. Select the four 'Critical' Priority 1 combinations from this table, absent local data.*
Sampling sites	*Depending on the objectives:* • *Hospital wastewater discharges* • *Inlets to wastewater treatment works* • *Nodes within the sewerage system (factoring in hydraulic residence times and high-risk inputs, such as hospitals)* • *Sentinel sites in non-sewered systems (i.e. gathering points, such as markets and places of worship)*

Sampling approach	*Depending on the objectives/targets:*
	• *Composite sampling methods are preferred*
	    ○ *using flow weighted automatic sampling*
	    ○ *compositing of serial grab samples*
	    ○ *passive 'Moore Swab' style samplers*
	• *Grab samples*

Transport and storage	*Conventional cold chain.*
Analytical methods	*Depending on the objectives/targets:* • *Culture-based to detect pathogens expressing the AMR phenotype of concern* • *Culture-enrichment and/or culture-independent directly extracted wastewater concentration, with ARG PCR to improve sensitivity, for detection of priority ARGs at lower levels* • *Sequencing to identify ARGs in circulation, potentially with selective culture and whole genome sequencing to enhance sensitivity*
Utilization of WES evidence	*Inform agencies responsible for:* • *Selection and administration of antimicrobials* • *Surveillance, prevention and control of the relevant disease* *Possible uses cases include:* • *Following AMR trends in the population* • *Early indication of introductions or increases in the prevalence of target AMR-pathogen combinations (e.g. increases in prevalence, new outbreaks, or failures of interventions)* • *Emergence of new AMR-pathogen combinations* • *Evidence of asymptomatic carriage in situations where there are no known clinical cases* • *Evidence of reduced local circulation following successful intervention* • *Evidence of the end of local circulation following outbreaks*

Contents

1 General information

1.1 Global burden and geographic distribution of antimicrobial resistance

The proportion of infections caused by AMR pathogens is increasing. During 2019, WHO identified AMR as one of the top 10 global public health threats facing humanity. Bacterial AMR was estimated as being directly and indirectly responsible for approximately 1 and 5 million global deaths, respectively.[1] By 2050 projections forecast this to almost double to being directly and indirectly responsible for approximately 2 and 8 million global deaths, respectively.[2] The global economic impacts of AMR have been estimated at up to US$3.4 trillion in lost gross domestic product by 2030 and US$1 trillion in additional healthcare costs by 2050.[3] The effects are particularly significant in low and middle income countries, and in sub-Saharan Africa. Longer term, given the benefits afforded by antimicrobial agents, it follows that the evolution of antimicrobial resistance (AMR; or AR) will partially reverse their health benefits. As bacteria, viruses, fungi and parasites evolve resistance, diseases become less amenable to treatment.

1.2 Zoonotic transmission and potential reservoirs

AMR is a 'One Health' challenge because many AMR pathogens and ARGs can be readily transferred to and from humans either directly, or via animals, and via environmental reservoirs and transfer pathways.[4–6] ARGs can be transferred between microorganisms within wastewater and environmental waters, wastewater treatment plant processes, and sludges. This means that ideally WES relating to understanding the circulation of AMR pathogens in humans would be undertaken in that broader One Health context of understanding their circulation in animals and the environment. The levels of non-human AMR pathogens and ARGs in wastewater and environmental waters can vary significantly between sampling sites and situations, due to both animal inputs and environmental sources. For pathogens that are restricted to human hosts, such as Typhoid and Paratyphoid *Salmonella* sp., animal and environmental sources are likely to be less confounding. It is very important to consider non-human inputs when interpreting the results of AMR WES. Non-human inputs from animals and environmental hosting (including in water and sewer microflora) may be particularly significant for sampling non-sewered settings, or sampling sewers with significant environmental water or animal waste inputs.

1.3 Human pandemic potential of AMR pathogens

Some microorganisms that have global or regional pandemic potential, such as respiratory viruses, are increasingly being treated with antimicrobials. Therefore, AMR is increasingly relevant to pathogens with a high human pandemic potential.

1.4 Role of antimicrobial agents

Antimicrobial agents include antibiotics against bacteria, and other antimicrobials, including antivirals, antifungals, and antiparasitics, that are administered to patients, but not sanitizers and disinfectants. They help to control infectious diseases by preventing and slowing the replication of pathogens, and/or by inactivating them. The development and appropriate use of antimicrobial agents proved to be one of the most effective public health interventions, with their use becoming routine from the middle of the 20th century.

2 AMR and wastewater and environmental waters

2.1 Potential inputs to wastewater and environmental waters

AMR pathogens and ARGs can arise from both human and animal sources, including from excreta and secreta, that feed into the catchments of wastewater and environmental water sampling points. In some cases AMR pathogens and ARGs can be present free-living within the environment, including within sewerage systems, wastewater treatment plants, and environmental waters. Indeed, many currently important clinical mechanisms of resistance may be evolved from environmental reservoirs. Environmental pollution by antimicrobials can select for AMR pathogens in those environments. These zoonotic and environmental hosts and harbourages of AMR microorganisms and ARGs, combined with the genetic mobility of ARGs, presents challenges for relating AMR WES to clinical contexts. The principal use case of relevance in the short term is descriptive, i.e. to provide baseline surveillance of occurrence over time in the broader environment. Over the medium to long term, however, possibly the strongest use case for AMR WES is to inform clinical or veterinary practice, such as the choice of antimicrobials preferred for treatment, and vaccination campaigns, hence to inform public health actions as well as to monitor the effects of interventions. It is too early to determine the relative value of AMR WES in smaller catchments to facilitate short term monitoring versus its use to monitor larger scale trends over longer periods of time.

2.2 Target persistence, degradation and risk of infectious AMR pathogens or transmissible ARG

AMR pathogens and ARGs exhibit sufficient persistence in wastewater and environmental waters that WES is demonstrably technically feasible.[7,8] The existence of analytical methods that can detect AMR pathogens and ARGs in wastewater and environmental waters permit WES to be used for these targets. However, due to the many sources of AMR pathogens and ARGs, interpreting the results from complex wastewater and environmental water systems in terms of human circulation is challenging. Furthermore, it is possible that the profile of AMR pathogens and ARGs changes, both within sewer networks and waterways, and within wastewater treatment plants, as ARGs are transferred between hosts For instance, some AMR pathogens may persist longer than others, and others may even replicate. Ongoing replication of microorganisms, particularly in biofilms, may result in potentially environmental sources of AMR pathogens and ARGs that are not related to shedding from infected humans. Therefore, these potential confounders need to be factored into sampling programs and results interpretation. For instance, it is critically important not to overuse and promote second- and third-line antibiotics in humans based on WES evidence of AMR to first-line antimicrobials without first understanding whether the WES data has clinical relevance, noting possible this possible confounding.

2.3 AMR WES experience

As summarized in Table 2, several pilot studies have compared AMR microorganisms and/or ARGs in wastewater and environmental samples to clinical samples in the relevant populations. Some of these studies found challenges relating the two sources of evidence, but others found acceptable and in some cases good correlations. Therefore, the use of WES to provide evidence of locally circulating AMR pathogens and ARGs in the human population is a proven but locally variable concept.

There is a growing number of AMR pathogens that have been cultivated and tested, or ARG that have been tested, and tested either directly or using sequencing, at scale from wastewater and environmental samples. Primarily these studies are of bacteria, but a growing body of evidence refers to fungi, parasites or viruses. Most bacteria studied are members of the widespread enterobacteriaceae family, such as *Escherichia coli*, that are numerous and readily cultivable from wastewater and environmental samples. Whilst such microorganisms can be pathogenic, and are important, the studies have typically not sought less common AMR pathogens.

Most studies have been short in duration and small in scale, with the results proving difficult to interpret from a public health perspective, and the results were not typically linked to tangible public health actions. Most studies were undertaken as pilots, to test for correlations between clinical and wastewater data, rather than to inform public health action. Further studies, particularly over longer timeframes, and in multiple contexts, and designed and undertaken to inform public health actions, are necessary to provide evidence of the potential to link AMR WES to public health actions.

Nonetheless, the WES studies undertaken to date demonstrate the technical feasibility of isolating AMR microorganisms and detecting and characterizing ARGs, either alone, or in combination with one another, and/or with other targets. The next step is to implement pilots of routine programs, and demonstrate the delivery of actionable results that inform beneficial public health interventions.

2.4 Role of AMR WES within a One Health context

This document covers the human health context. However, as noted above, to be of greatest value, any surveillance program relating to AMR needs to be undertaken within a 'One Health' context since the AMR pathogens and ARGs can circulate between human and animal hosts, and environmental habitats.[4]

2.5 Relevant evidence from environmental monitoring studies

Whilst not undertaken for WES, or to inform public health interventions, the results of wastewater and environmental water monitoring studies carried out for other purposes can provide some evidence to predict the potential operational viability of AMR WES.[9,10] These environmental monitoring studies may have been undertaken to assess AMR in the environment as distinct from testing to understand public health and clinical relevance. AMR pathogens and ARGs have been monitored in wastewater and environmental waters for decades, with a variety of methods proven for such purposes. AMR pathogens and ARGs are routinely detected globally. Both can be monitored together as part of multi-target monitoring, and in association with other pathogens, such as other bacterial pathogens. These studies are seeking to understand what AMR pathogens and ARGs are being shed into that wastewater as part of understanding the risk of spreading AMR pathogens.

3 AMR surveillance overview

3.1 Purpose of AMR surveillance

AMR is exacerbated by evolutionary selection pressure on pathogens arising from the use of antimicrobials. Selective targeting and utilization of antimicrobials can help to reduce the selection pressure that promotes AMR. Evidence of AMR can be fed back into clinical practice guidelines and standards. Decisions on when to use antimicrobials, which ones, and in what context, can be influenced by that evidence. The aim is to find a balance between the immediate needs of a patient to be treated with antimicrobials and the broader public health need of helping to reduce the evolutionary pressure that increases AMR. With such a genuine tension, the best available evidence can inform the best clinical and public health decisions. There are multiple global, regional, and national AMR surveillance systems aimed at providing that information.

3.2 Examples of global AMR surveillance programs and the context of WES

As part of the Quadripartite WHO-FAO-WOAH-UNEP Global Action Plan to tackle AMR (GAP-AMR), approved by all WHO Member States in 2015, the Global Antimicrobial Resistance and Use Surveillance System (GLASS) was launched to collect and report epidemiological, clinical, and population-level data (https://www.who.int/initiatives/glass). Foundational steps in managing AMR include national surveillance to generate good-quality data to inform action.[11] This evidence includes clinical information, antimicrobial use data, and broader research. WES can provide additional evidence to support this foundational step, which can be used to support public health monitoring and control actions.[4]

Since 2018 WHO has undertaken a One Health module for integrated multi-sectoral surveillance of extended-spectrum beta-lactamase (ESBL)-*E. coli* (Tricycle) (https://www.who.int/initiatives/glass/glass-modules-7). This involves testing ESBL-*E. coli* in samples collected from human, poultry, sewage, and environmental water (market runoff and river sites in urban areas). The protocol has been piloted in nine WHO Member States (Ghana, Indonesia, Madagascar, Malaysia, Pakistan and Senegal, India, Jordan and Nepal) and is being implemented in five others (Zimbabwe, Zambia, Nigeria, Iran and Morocco).

Within this broader context, AMR WES has the benefit that it is potentially more representative, and not limited by diagnostic access or healthcare utilisation, and more cost-effective, compared with clinical isolate/individual-level surveillance. However, at present it is not a core part of such surveillance programs. Rather, at the time of writing, evidence used to inform decisions on antimicrobials is typically derived from surveillance of AMR in samples collected from patients that experience infections with AMR pathogens, and from evidence of use of antimicrobials. WES can provide additional population-level evidence of the presence of AMR pathogens and genes in wastewater and environmental waters. This WES evidence can potentially be utilised along with other evidence to help decide which antimicrobials are most likely to be effective at the clinical decision-making level, and to monitor spatial and temporal patterns of AMR to help assess how the use of antimicrobials and other contributing factors is influencing AMR to inform AMR minimisation strategies. However, at present, there is not enough that community-level WES evidence can be directly linked or reliably correlated with clinically relevant AMR from humans. Therefore, decisions on antimicrobial prioritisation are best made in a cross-sectoral context, factoring in animal pathogens, commensals, and environmental confounders. The objectives of influent monitoring defined by EU-WISH/JAMRAI2 includes monitoring of population level AMR trends, and detection of emerging AMR variants with public health concern.

3.3 Examples of country AMR surveillance programs

For context, whilst not directly related to AMR WES, it is important to understand where AMR WES might fit in with existing broader AMR surveillance programs. Some examples of such broader AMR surveillance programs are listed here, some of which include some pilots or proposed AMR WES components.

The United States Centers for Disease Control and Prevention (CDC) is part of the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) (https://www.cdc.gov/narms/about/index.html). The CDC's focus is on AMR studies of human isolates of *Salmonella*, *Campylobacter*, *Shigella*, *E. coli* O157, and non-cholera *Vibrio*. In parallel, the Food and Drug Administration (FDA) conducts serotyping, AMR phenotyping, and/or genotyping for *Salmonella*, *Campylobacter*, *Enterococcus*, and *E. coli* from retail meat and seafood samples (e.g. chicken, turkey, beef, pork, shrimp, tilapia, and salmon). Finally, the United States Department of Agriculture (USDA) conducts similar tests on isolates of *Salmonella*, *Campylobacter*, *Enterococcus*, and *E. coli* from food-producing animals with samples collected from slaughtering and meat processing plants. So whilst this program covers clinical, veterinary, and meat samples, a WES component is not included. However, WES for AMR is being looked by the Division of Healthcare Quality Promotion who are funding pilot surveillance efforts in multiple US states to evaluate utility. The National Wastewater Surveillance System provides a potential means of undertaking AMR WES as part of a multi-target programme (https://www.cdc.gov/nwss/index.html).

The European Centre for Disease Prevention and Control (ECDC) has conducted the European Antimicrobial Resistance Surveillance Network (EARS-Net) (https://www.ecdc.europa.eu/en/about-us/networks/disease-networks-and-laboratory-networks/ears-net-data). The program has been in operation since 1998 draws blood and cerebrospinal fluid samples and tests for AMR profiles of *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium*. This program covers invasive clinical samples, but a WES component is not included. However, testing for AMR in both wastewater treatment plant influent and effluent is required under the Urban Wastewater Treatment Directive, which is expected to rapidly provide improvements in methods and data and lead to an improved understanding of what evidence WES can provide and how it can be used to inform policy.

The Canadian Centre for Foodborne, Environmental and Zoonotic Infectious Diseases (CFEZID), Infectious Diseases and Vaccination Programs Branch (IDVPB), Public Health Agency of Canada (PHAC), and One Health Division, and Division of Enteric Diseases, National Microbiology Laboratory Branch (NML), of PHAC, have coordinated the Canadian Integrated Program on Antimicrobial Resistance Surveillance (CIPARS) (https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/about-cipars.html). Since 2002 the program has collected, analyzed and communicated trends in antimicrobial use and AMR for select bacteria from samples from humans, animals, and retail meat across Canada. The program targets *Salmonella* spp. and *Campylobacter* spp. in samples from humans and food animals, *E. coli* from food animals, and interrogation of samples from symptomatic food animals (cattle, pigs, horses and poultry). Integration with the sales of antimicrobials is part of this program, but WES is not included.

The Australian Commission on Safety and Quality in Health Care undertakes the Antimicrobial Use and Resistance in Australia (AURA) that was established in 2015 (https://www.safetyandquality.gov.au/our-work/antimicrobial-resistance/antimicrobial-use-and-resistance-australia-aura). This is focused on healthcare facilities and does not include a WES program. Targets include methicillin resistant *Staphylococcus aureus*, ciprofloxacin resistant *E. coli*, and vancomycin resistant *Enterococcus faecium*. In addition, notifications from clinical samples are collated through the National Alert System for Critical Antimicrobial Resistances (CARAlert) for carbapenemase-producing *Acinetobacter baumannii* complex (*A. baumannii, A. calcoaceticus, A. dijkshoorniae, A. nosocomialis, A. pittii* and *A. seifertii*), *Candida auris*, Carbapenemase-producing, and/or ribosomal methyltransferase-producing, and transmissible colistin resistant *Enterobacterales*, linezolid-resistant *Enterococcus* spp, multidrug-resistant – resistant to at least rifampicin and isoniazid – *Mycobacterium tuberculosis*, ceftriaxone- and/or azithromycin-nonsusceptible, or gentamicin-resistant *Neisseria gonorrhoeae*, ciprofloxacin-nonsusceptible *Neisseria meningitidis*, carbapenemase-producing *Pseudomonas aeruginosa*, ceftriaxone-nonsusceptible *Salmonella* spp., multidrug-resistant *Shigella* spp, vancomycin- or linezolid-nonsusceptible *Staphylococcus aureus* complex (*S. argenteus, S. aureus* and *S. schweitzeri*), and penicillin-reduced susceptibility *Streptococcus pyogenes*. The program is integrated with data on community antimicrobial use from prescription data.

The Singaporean National Strategic Action Plan on AMR, and surveillance reports (https://www.ncid.sg/Health-Professionals/Pages/Antimicrobial-Resistance.aspx), incorporates all relevant sectors to cover One Health sectors, including WES studies, are reported every two years (https://isomer-user-content.by.gov.sg/18/32ddbb35-dd6a-4619-aa32-aaa5bd87a79c/NSAPv2_Final_12Nov2025_for%20publication.pdf)

4 WES for AMR, objectives and related public health actions

4.1 Experience with WES for AMR

Monitoring of AMR for reasons other than WES

As noted above, monitoring of wastewater, sludge, and environmental waters has been undertaken for reasons other than WES. These reasons include for environmental monitoring and environmental protection purposes. For instance, wastewater and solid waste may be monitored as part of informing the control of residuals and wastes arising from the manufacturing of antimicrobials[12], or as part of understanding the environmental spread of AMR and antimicrobials in wastewater, and the risks that these water and sludge matrices may pose through exposure to humans, animals, or the environment.[13] However, these applications are not necessarily related to understanding the AMR profiles of pathogens infecting humans, hence they are not undertaken for the same objectives as AMR WES. Nonetheless, the sampling and analytical methods, and some of the data, can be repurposed for AMR WES use cases.

WES for AMR

Whilst WES for AMR is not as widely and routinely practiced as WES for poliovirus or SARS-SoV-2, there are sufficient studies to provide proof-of-concept of its use for specific use cases.

A systematic review of WES studies, explicitly testing for concordance between AMR prevalence in clinical samples and AMR WES results, found reasonably high concordance (correlation coefficients above 0.8) of wastewater-human AMR prevalence estimates among 24 studies that met the inclusion criteria for further analysis.[7] Most studies used a combination of phenotypic and genotypic characterization. Many of these studies focus on healthcare facility sampling, however. Indeed, most WES AMR experience comes from healthcare facilities, that have been targeted directly for WES studies, with samples collected from sample points representative of a facility's wastewater. A systematic review of 37 such studies reported evidence of higher levels of certain types of AMR in hospital wastewater compared to community wastewater in 80% of studied.[8] However, the lack of standard methods of sampling, analysis, resulted in a range of probably biases between studies that precluded comparison and meta-analysis.

In principle, WES has been described as providing cost-effective evidence to help inform the prevention and management of infections in healthcare facilities, including the selection of antimicrobial agents, and to help with early detection of outbreaks.[14] However, there is no established guidance or decision-support tool for designing and interpreting such AMR WES programs for healthcare facilities. As a result, decisions on sampling locations, methods, frequency, analytical targets, and interpretation, need to be made for specific sites and situations, rather than by following proven guidance. Complications noted with WES for AMR include the challenges of capturing representative samples, and the potential for AMR profiles to change due to biofilms in water and wastewater systems, particularly as the sampling sites become more remote from the point of discharge to the wastewater. Consistent relationships between AMR pathogens and ARGs detected in healthcare facility wastewater have yet to be reliably correlated to clinically relevant infections in patients. Additional biases that can be introduced arise from varying methods (sampling, storage/transport, extraction, sequencing, bioinformatics, analysis), which need to be resolved via standardization or accounting for those biases.

Ways forward for the work required locally, to set the foundations for a useful WES AMR study for a healthcare facility, include:[15]

- understanding wastewater flows within the facility to help determine both when and where to sample to gather evidence from the targeted patients and catchments of interest;
- identification of safely accessible sampling points by factoring in practical considerations to determine feasibility;
- aligning the timing and the target pathogen-antimicrobial combinations from both the wastewater and the clinical sampling and analysis activities to enable correlations to be assessed between WES and clinical results to help with their interpretations; and
- full validation of the assays undertaken to ensure adequate sensitivity and specificity for the context.

4.2 Routine WES AMR targets

Public health priority pathogen-antimicrobial combinations

Prioritization of targets for routine monitoring requires understanding priority pathogen-antimicrobial combinations. However, there are hundreds of pathogens, and hundreds of AMR profiles. This makes narrowing the scope of and prioritizing an AMR WES program challenging. This is further complicated by global and regional variations in priorities. Therefore, the first stage in developing an AMR WES program needs to be targeted to local priorities, covering a manageable number of pathogen-AMR combinations. This choice is also influenced by the use case, i.e. the purpose of the program. A good starting point is the country or regional National Action Plan on AMR, and the WES AMR prioritization should likely align with those priorities.

As a general principle, WES for AMR is best focused on pathogen-antimicrobial combinations and sampling and analysis strategies that are most likely to provide information actionable for public health.[4] Therefore, a good starting point is to set up the AMR WES priorities to be complementary to existing clinical surveillance priorities. This ensures that the target pathogen-AMR combinations are established priorities, as well as being more likely to have an established public health surveillance and management framework within which to help design the WES program, and utilize the reported data.

Globally, priority pathogen- antimicrobial combinations for further research have been previously identified by WHO for bacterial pathogens, building on previous prioritization efforts.[16] These priorities have not been set specifically for WES, but, as noted above, they do provide a useful starting point for aligning WES with those broader surveillance priorities. A summary of these priorities is given in Table 2, along with additional information on the possible role of WES, and of the status of demonstrated WES applications.

Table 2. Examples of priority research target pathogen-antimicrobials combinations and notes on the possible role for WES.

WHO global priority pathogens list of antibiotic-resistant bacteria[16]	Potential role for WES**	Examples of WES studies
Priority 1: CRITICAL		
Acinetobacter baumannii, carbapenem-resistant		[17]
Enterobacterales, third generation cephalosporin-resistant*		[18,19]
Enterobacterales, carbapenem-resistant*		[20,21] Blaak
Mycobacterium tuberculosis, rifampicin-resistant		[22]
Priority 2: HIGH		
Salmonella Typhi, fluoroquinolone-resistant		
Shigella spp., fluoroquinolone-resistant		
Enterococcus faecium, vancomycin-resistant		[17,20,23,24,25]
Pseudomonas aeruginosa, carbapenem-resistant		[26]
Non-typhoidal Salmonella, fluoroquinolone-resistant		
Neisseria gonorrhoeae, third-generation cephalosporin, and/or fluoroquinolone-resistant		
Staphylococcus aureus, methicillin-resistant		[17,20,27]
Priority 3: MEDIUM		
Group A streptococci, macrolide-resistant		
Streptococcus pneumoniae, macrolide-resistant		
Haemophilus influenzae, ampicillin-resistant		
Group B streptococci, penicillin-resistant		
Other global priorities		
Mycobacteria (including Mycobacterium tuberculosis and non- tuberculosis Mycobacteria)		[22]
HIV		
Plasmodium spp. malarial parasites		
Additional local and regional priorities		
To be completed by local and regional WES teams		
(for instance Candida auris has been included in some studies)		

*This order of bacteria includes both *E. coli* and *Klebsiella pneumoniae*.
**This would be context-specific, to be completed by local and regional WES teams.

Considerations would include local prevalence, how organisms are shed (noting that sewage is mostly gut flora, so may contain less skin or respiratory tract associated microorganisms), and how the target persists and changes.In terms of health impact, it was estimated that in 2019 just six pathogens accounted for the majority (73.4%) of deaths attributable to bacterial AMR globally, each being associated with more than 250,000 AMR-related deaths, in priority order:[1]

- *E. coli*
- *Staphylococcus aureus*
- *Klebsiella pneumoniae*
- *Streptococcus pneumoniae*
- *Acinetobacter baumannii*
- *Pseudomonas aeruginosa*

Six more pathogens were each responsible for between 100,000 and 250,000 deaths associated with AMR, in priority order:

- *Mycobacterium tuberculosis*
- *Enterococcus faecium*
- *Enterobacter* spp.
- *Streptococcus agalactiae* (group B *Streptococcus*)
- *Salmonella* Typhi
- *Enterococcus faecalis*

The pathogens directly causing the most deaths from AMR were, in priority order:

- *E. coli*
- *Klebsiella pneumoniae*
- *Staphylococcus aureus*
- *Acinetobacter baumannii*
- *Streptococcus pneumoniae*
- *Mycobacterium tuberculosis*

The deadliest pathogen-antimicrobial combination was methicillin-resistant *Staphylococcus aureus* (more than 100,000 attributable deaths), with another six pathogen-antimicrobial combinations causing between 50,000 and 100,000 deaths:

- multidrug-resistant excluding extensively drug-resistant tuberculosis
- third-generation cephalosporin-resistant *E. coli*
- carbapenem-resistant *Acinetobacter baumannii*
- fluoroquinolone-resistant *E. coli*
- carbapenem-resistant *Klebsiella pneumoniae*
- third-generation cephalosporin-resistant *Klebsiella pneumoniae*

WES priority pathogen-antimicrobial combinations

The evidence on priority pathogen-antimicrobial combinations has helped to prioritize improvements in infection prevention and control, and development and deployment of alternative antimicrobials and vaccines. However, whilst this global evidence based on 2019 data provides a useful starting point, these priorities are changing over time, and will differ between countries and regions. Furthermore, this public health priority does not necessarily correlate to the WES priority. For instance, if there is ample evidence from clinical samples due to high levels of local circulation of an AMR-pathogen combination, WES may provide limited actionable evidence beyond that already available from clinical data.[4]

WES can provide useful evidence as a research tool to assist in understanding the spatial and temporal patterns of these targets. Because samples represent whole communities, WES can potentially be most cost-effective when targeting low-prevalence AMR pathogens of high public health relevance[4] that might not be known to be locally circulating, or where there is interest in understanding spatial and temporal patterns and trends in their circulation.

Evidence from WES will not provide such tangible information on disease burdens and deaths, but it can potentially provide evidence on spatial and temporal AMR pathogens and ARGs circulating within human (and potentially animal) populations that contribute to sampled wastewater and environmental waters.

Routine WES has a theoretical potential to provide evidence to fill gaps where which there is limited clinical data. In the long term, this may potentially be useful to provide:[4]

- an early indication of introductions or increases in the prevalence of target AMR-pathogen combinations (e.g. increases in prevalence, new outbreaks, or failures of interventions);
- emergence of new AMR-pathogen combinations;
- evidence of asymptomatic carriage in situations where there are no known clinical cases;
- evidence of reduced local circulation following successful interventions; and
- evidence of the end of local circulation following outbreaks.

In all cases, the spatial and temporal sampling strategy, and the sensitivity and specificity of the WES monitoring assay used, needs to be good enough for its intended use. Public health agencies responsible for monitoring and managing AMR, and making decisions on the use of antimicrobials, can set priorities for WES programs to help fill evidence gaps and inform public health actions. Keeping a watching brief on method development can help to move with the latest innovations. For instance, whilst most genomic/molecular based WES is currently optimised in future for looking for known genetic signatures , metagenomics of wastewater could be used to detect the ingress of novel genetic signatures (i.e. for threat detection.

4.3 Agile (or responsive) WES for AMR

Agile WES responses can be aligned with those from other clinical, veterinary and food, etc., surveillance programs. The WES program can ramp up in response to the detection of concerning pathogen-AMR combinations from any sample type, including the routine WES program, and other surveillance.

4.4 Potential use cases and public health actions from the addition of WES for AMR

At present, linking AMR and ARG WES to public health actions remains a conceptual rather than operationally proven use case. In principle, the population-level public health actions taken in response to evidence from WES would be analogous to those triggered by the synthesis of results from clinical surveillance programs. This includes:

- Infection control: Public health interventions, such as improved WASH, facility maintenance, and infection control practices, could be promoted more strongly and enhanced where evidence from WES indicated elevated AMR in the human population in an area.[13]
- Trending: Providing ongoing community-level surveillance data to monitor the AMR pathogens and ARGs in circulation and have them trended and compared over time and space. This evidence could, potentially, be used to help inform long-term decisions on the preferred antimicrobial agents to be used, provided the AMR WES evidence was sufficiently specific to human shedding and could discriminate animal and environmental AMR.
- Diagnostic testing: Clinical antimicrobial susceptibility tests, and other diagnostics, could be prioritized towards the pathogen-AMR combinations that are most prevalent in an area based on WES evidence.[11]
- Selecting preferred antimicrobials: The use of one or more antimicrobials could be selected against, in favor of alternatives, if there were local evidence from WES of high levels of a particular pathogen-antimicrobial combination, or high levels of resistance to particular antimicrobials.[28]

- Development of new antimicrobials: Research and development could be prioritised to develop novel antimicrobials in areas where WES evidence indicates that resistance to existing antimicrobials is at high levels.[16]
- Vaccination programs: Vaccine development, and vaccination administration, could be prioritised and increased, to target populations, based on local WES evidence of high levels of AMR pathogens in an area, noting that those infections will be more difficult to treat.[11]
- Early warning: WES could be used to detect the 5mergence of AMR properties, either due to novel mutations, or transfer of ARG between hosts, or of AMR pathogens or ARGs that move across borders. For instance, WES has potential to detect early ingress of known carbapenemase genes into a defined setting, such as long-term care facilities or hospitals, over short timeframes. AMR is typically a problem that builds steadily, over long timeframes, rather than rapidly, and not usually within the periods of weeks. Even in those cases WES can provide early warning relative to clinical detection methods.

5 WES additional methodological considerations for AMR

This section should be read in conjunction with general methodological consideration in Section 5 of *Wastewater and environmental surveillance for one or more pathogens: Guidance on prioritization, implementation and integration* (available here).

The choice of sampling strategy, sample locations, sampling and transport methods, and laboratory and bioinformatic analytical methods can all significantly influence the AMR and ARG profiles from the WES program. Therefore, these factors need to be considered carefully, and design optimised, to best provide information to support the broader public health objectives to which the WES program is contributing.

5.1 Sampling approaches

A diverse range of sampling methods have been shown to be effective for AMR for WES, including single grab, composited grab, proportional composite, and passive.[7,8] Similarly, a diverse range of sampling point types have been successfully utilized, including sampling of effluent from high-risk facilities such as healthcare facilities and abattoirs, from accessible points within sewerage networks, and at wastewater treatment works (WWTW) inlets. Timeframes for sampling have varied between one-off to longer term campaigns. The timing of the wastewater sampling has been independent of, or timed to align with, the collection of clinical samples.

A review of these various approaches for sewered systems noted that composite sampling, of WWTW influent, longitudinally over one or more years, with timing and location aligned to clinical sampling, provided the strongest correlations between WES and clinical samples at the population-level.[7] Therefore, if the objective is to correlate the two, WES would typically be undertaken using time-/location-matched sampling of wastewater with clinical samples, using composite sampling, sampling from WWTW influent, for periods of one or more years to help understand the impacts of seasonality. For research projects and pilot studies, this requires collaboration with clinical surveillance agencies to permit temporal alignment of wastewater and clinical sampling. For both research and pilot projects, and for routine use of WES, this requires collaboration with sanitation agencies to enable an understanding of the spatial and temporal aspects of sewer catchments and to provide access to sample points. This can be more challenging in settings where there are multiple separate wastewater agencies involved in providing sanitation services to a study area. For smaller catchments, such as healthcare facilities, passive samplers may be preferred.[29] To meet other sampling objectives, such as broader sampling to understand the AMR profiles of the community, so broader than just from healthcare facilities, AMR WES samples would not necessarily need to align with clinical samples, and it may be more informative when the AMR WES and clinical programs are undertaken independently.

Sampling in treated wastewater has been commonplace in non-WES AMR pathogen and ARG monitoring of wastewater because there is interest in what is being discharged to the environment. However, this data is of limited value in the WES because biological wastewater treatment processes change the AMR profile of the microbial population.[30,31] Similarly, in sewerage systems with long transport periods, the AMR profile of the microbial population may change during transport. Therefore, sampling for AMR WES should take place at the WWTW influent (and not the effluent), with the WWTW influent sampling site alone being sufficient for smaller sewerage systems, as well as at sentinel sites upstream of the WWTW within the wastewater catchment for larger sewerage systems.

AMR pathogens and ARGs can be shed by infected, as well as infected animals, and can be presented in the environment, which can lead to potential confounding of results. A noted in the overarching document in this series, normalization methods, such as using phage or bacteria that are more specific to humans, can assist with interpreting results of mixed waste or environmental water streams. Even then, it may not be possible to determine whether the origin of any AMR pathogens or ARGs found in wastewater or environmental water samples is from infected humans or other animal or environmental sources. This has led to the untested theoretical hypothesis that WES results would be most actionable for samples collected at facility scale, such as from hospitals, where non-human inputs are likely to be minimized.[32]

On the other hand, whilst sampling of specific high-risk facilities, such as from hospital wastewater is useful for facility-scale studies, it may be of limited value in providing evidence related to the profiles of pathogens circulating in the broader community. The pathogen-AMR combination and ARG profiles of high-risk sites, such as hospital wastewater, is often very different to that of the general population.[33] For instance, hospital settings only capture persons that have sought medical attention and been admitted.[4]

Facility-scale sampling is appropriate if the objective is to understand pathogen-antimicrobial combinations or ARGs circulating within the population using that facility, or the contribution the facility is making to wastewater pathogen-AMR burdens. Broader-scale sampling is required to understand pathogen-antimicrobial combinations or ARGs circulating within the general population. However, it may be important to separate the two and sample both sites since differences between the two have been reported.[8] Separating sampling in this way helps to examine whether pathogen-antimicrobial combinations or ARGs found from community-scale sampling are coming from the wider community versus being limited to facility settings. Furthermore, community-scale settings may be less sensitive and less able to detect antimicrobial combinations or ARGs that are limited to facilities. Therefore, in practice, there may be good reasons to separately collect samples representative of facilities as distinct from community-level sampling of the wider population.

Transport and storage of samples at ambient, cold-chain, and below freezing temperatures, can all introduce their own biases.[34] As the default, however, cold-chain remains the most comment method used. There may be changes in AMR composition during ambient storage due to biological activity, and there is the risk of introducing non-cultivability for some microorganisms if they are transported and stored at refrigeration temperatures. Freezing samples before thawing and analysis has been found to modify the cultivability of some microorganisms, thereby introducing a bias. The influence of transport and storage temperature can be further investigated.

5.2 Laboratory and analytical methods and interpretation

The choice of concentration, extraction, bioinformatic and analytical methods can influence the AMR and ARG profiles detected and reported in tested wastewater samples. A wide range of methods and approaches can be used to extract target microorganisms and/or their nucleic acid to test susceptibility to antimicrobials and establish AMR profiles, including[29,35–37];

- cultivation methods:
    - disc-diffusion
    - serial dilution in broth
    - selective media
    - chromogenic media
- direct genomic methods:
    - targeting specific genes in wastewater directly using targeted qPCR, dPCR
    - metagenomics
    - hybridization-capture/probe-based and sweep metagenomics
    - Selecting suitable sequencing methods (e.g. short versus long)
- combined methods that undertake follow up analysis of cultured microorganisms using:
    - targeted PCR, qPCR or dPCR

Whilst culture-based methods to test AMR phenotype have been the primary analytical approach used in many historical studies, these methods will be biased in favor of the subset of microorganisms that can be most readily cultivated.[38] When cultured microorganisms are tested for AMR properties, by testing for inhibition of their growth in vitro, this provides evidence of expression of the AMR phenotype for a specific pathogen. Follow up genetic testing can be used to characterize the AMR isolates.

More recently, studies are increasingly using metagenomics, including both short and long read approaches , to detect ARGs and their associated characteristics. However, the relationship between ARGs found in WES samples, and their potential hosts and clinical relevance, is unclear.[29] Targeting of specific ARG using PCR has been undertaken, but is of limited value, other than to identify the target ARG in wastewater or environmental water samples. The implications for public health action are limited to scenarios where that action is coupled to evidence of the presence or absence of a specific ARG of interest, rather than where evidence is required of its expression within a virulent pathogen leading to phenotypic AMR properties.

In practice, testing for ARGs alone is of limited value for AMR WES to assess immediate public health relevance. The very mixed nature of the WES samples means that merely finding ARGs in the sample does not directly indicate that:

- the ARGs arose from human sources (they could have arisen from animal or even environmental sources, including the microflora of the water or sewer environment);
- the ARGs are associated with any one viable and clinically relevant organism, or are in the combination within one cell or pathogen, as required to confer AMR properties on a pathogen (rather than being dispersed across multiple microorganisms in the sample);
- those ARG are likely to be expressed sufficiently to confer resistance upon their host;
- other genes are present, or expressed, that are required for AMR to be clinically relevance, such as the permeability, efflux, and modification of the antimicrobial;
- other intrinsic co-factors are present, (if required), for the AMR phenotype to be conferred, or for the potential pathogen to be virulent;
- ARG are present within a potentially pathogenic species as distinct from a non-pathogenic species; and
- additional virulence factors required for a pathogen to infect and cause illness in humans are present.

In all cases, there are no standardized methods, and there is a wide diversity with respect to what AMR pathogens and/or ARG are sought, and what methods are used.

5.3 Reporting and communication

Ideally, WES for AMR pathogens and ARGs would be reported and communicated as part of the broader global, regional, national, and sub-national surveillance, monitoring, and management programs, as discussed in Section 6.

5.4 Acceptability of WES for AMR

There is no evidence of any special considerations that may deter public acceptability of WES for AMR. On the contrary, the European Commission Urban Wastewater Treatment Directive (https://eur-lex.europa.eu/eli/dir/2024/3019/oj/eng) incorporated the need to monitor AMR at large wastewater treatment plants. This demonstrates acceptance, in principle, of AMR WES at scale. However, it is possible that metagenomics could capture human DNA sequences, which may be relevant for wastewater from small-scale catchments, and using long-reads. This may create a low risk that human genetic information relevant to individuals is captured, and even potentially made publicly available.

6 Integrated surveillance and multitarget WES considerations

6.1 Integration of AMR WES into existing surveillance and response

Similar to section 8.3, ideally, WES for AMR pathogens and ARGs would be integrated into the broader global, regional, national, and sub-national surveillance, monitoring, and management programs, as discussed in Section 6.

6.2 Integration of multi-target WES together with AMR

There are no established routine operational AMR WES programs demonstrating integration of AMR and/or ARG integration as part of multi-target WES, or within existing multi-modal public health surveillance programs linked to public health action. However, successful proof of concept pilot studies have demonstrated the technical feasibility of the monitoring component of such an approach. This includes monitoring both AMR and ARG together, and along with other WES targets.

7 Key knowledge gaps and applied research priorities

Key questions to test with future research including the following:

- What pathogen-AMR combinations are the highest priority targets for each setting?
- What are the preferred sampling, analysis and bioinformatics workflows and how sensitive and specific are they, including method validation on simulated samples and data?
- What are the demonstrated health-impactful use cases to respond to WES evidence with respect to AMR?

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