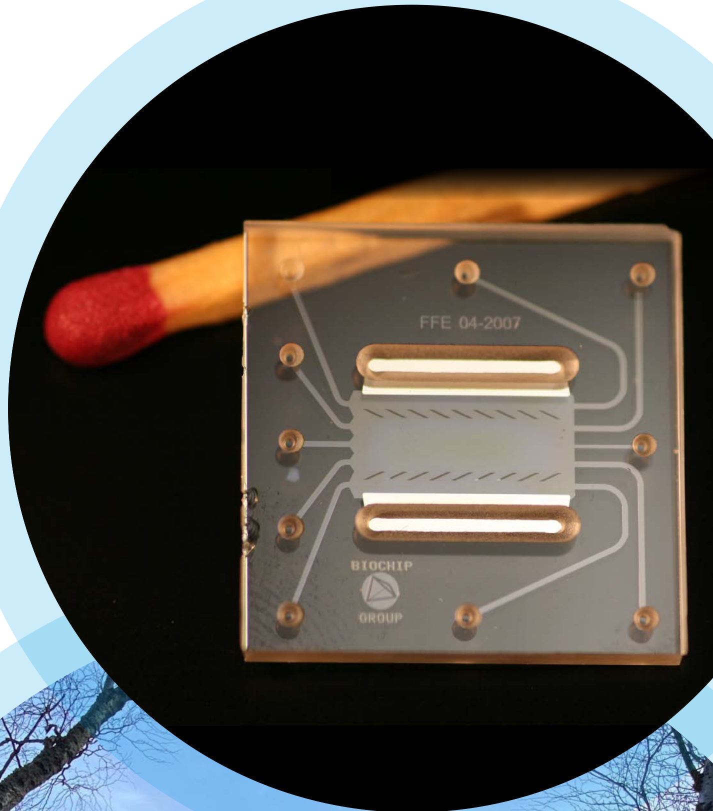
CREW CENTRE OF EXPERTISE FOR WATERS

Technologies for Monitoring and Treatment of Antimicrobial Resistance in Water

CREW Policy Brief



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Technologies for Monitoring and Treatment of Antimicrobial Resistance in Water

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Foreword

Implementing antimicrobial resistance (AMR) surveillance is critical to understand the role of water in proliferation and transmission of AMR, the risks to human health and the environment, and to inform selection, and application, of mitigation methods and treatment options.

This policy brief addresses current issues regarding methods for monitoring and treating AMR in water, covering existing and emerging technologies, and proposes seven key policy recommendations to address the challenge of AMR in water in Scotland.

Overview

- At present, there is no consensus on the best approach for monitoring or treating AMR in Scotland's Waters.
- Emerging monitoring technologies include lab-on-a-chip, sensors and miniaturised sequencing platforms.
- A Code of Practice is required to enable technology developers to understand different use cases, key measurement parameters, and validation methods, to drive future improvements in detection systems.
- Improved risk assessment methodologies are essential to effectively utilise monitoring data.
- There is a need to better understand the performance of different treatment technologies, including cost-benefit analyses to enable comparisons, taking into account wider implications in the environment.

Background

The UK 5-year National Action Plan for Antimicrobial Resistance (UK NAP) 2019-2024 highlights the importance of the role of the environment, including water, and taking a One-Health approach to tackling antimicrobial resistance (AMR). Our CREW Policy Note* describes the current situation in Scotland, outlining the status of AMR in Scotland's waters.

Reducing the spread of AMR in the environment is a significant challenge, for which implementing environmental surveillance is critical and urgently needed (UN Environment Assembly, 2017). A summary of the environmental dimensions of AMR, including the need for a One Health approach, is given in a recent policy maker report by UN Environment Programme (UNEP, 2022).

Multiple studies have established links between drivers of AMR (e.g. heavy metals, pharmaceuticals etc., released into water) and the dissemination and transfer of AMR in water, especially with poor removal in wastewater treatment plants. However, there is still much work to be done to fully understand the mechanisms of AMR dynamics in water as well as to work out the best approaches to tackle this challenge.

One particular issue for AMR surveillance in the water environment is the lack of consistency in approaches and methods. There is no consensus on what we should measure, what the best approaches to measurement are and what the actionable steps monitoring results should trigger are.

What are the existing methods of detection for antimicrobial resistance?

There are two main approaches to detection of AMR and antimicrobial resistance genes (ARGs):

- Phenotypic methods, where detection relies on growth of the bacteria in the presence of antibiotics; susceptibility is measured by the ability of those antibiotics to inhibit growth using various approaches. With the addition of biochemical assays, e.g. using efflux pump inhibitors, further information can be inferred regarding the mechanism of resistance, suggesting which ARGs might be present.
- Genotypic methods, which analyses extracted DNA and can be divided into two main approaches: (i) utilising DNA amplification techniques to detect specific targets (i.e. known ARGs) and (ii) adopting genomic methods such as whole genome sequencing or metagenomics to identify ARGs without pre-defining specific targets.

Find out more: For more details on the methods see the section of the full literature review entitled "Review of methods to detect AMR in the environment and the associated biases" (Appendix A).

Box 1: Definitions

AMR – antimicrobial resistance: this is when microbes evolve to become more or fully resistant to antimicrobials. The development of drug-resistant microbes is recognised as a global public health threat. Global deaths due to AMR are estimated at 700,000 per year at present, with predictions that this figure could rise to millions in the near future. AMR is a significant burden on healthcare systems and society – in the EU healthcare costs and productivity losses due to AMR are estimated at €1.5billion annually.

ARG – antimicrobial resistance genes: a wide range of genes have been identified which confer different aspects of resistance. ARGs can be transferred between bacteria through mechanisms such as horizontal gene transfer. Therefore, human and animal pathogens can acquire resistance from other bacteria.

*Avery, L., Pagaling, E. and Bridle, H (2022). Water and Antimicrobial Resistance in Scotland - status and solutions : CREW Policy Note. ISBN 978-1-911706-05-2. Scotland's Centre of Expertise for Waters (CREW). Available online at: https://www.crew.ac.uk/publications)

What are the main challenges in developing new technologies for antimicrobial resistance detection?

Existing techniques are often implemented in specialised laboratory facilities by skilled technicians. The focus of a lot of emerging technologies is on translation of existing approaches into more user-friendly, low-cost formats, e.g. automated and miniaturised systems. However, some of the key challenges are:

1. Many detection methods, from existing commercially available tools to recently published academic advances, have been developed for clinical diagnostics to guide treatment decisions. Given this primary aim is distinct from the goals of environmental surveillance, monitoring approaches are not always transferable
2. There is significant complexity in environmental samples, e.g.
    - Highly variable amounts of ARGs found, for which there is no technique at present capable of detection across this whole range ;
    - Even within one resistance gene (Box 1), there is likely to be diversity in the sequence, which detection methods need to be able to cope with;
    - the presence of other components (e.g. inhibitors) in water which impact on detection technique performance, that vary over time and between different water types
3. There is considerable variation in the protocols even within one test method making comparisons between test approaches and results difficult

Ideally, standardised protocols should be identified to enable reliable and comparative monitoring, as well as to inform development of new technologies. However, this is not yet possible as there is no consensus on the best approaches. While different monitoring goals might require the use of different techniques or protocols, there is a need for a set of standard targets, and agreed metadata information (e.g. flow rate, temperature, pH), which can easily be incorporated into different research or monitoring projects to generate further comparative data.

Furthermore, the link between monitoring data and risks needs to be better understood, particularly the relationship between ARG measurements in the environment and clinical/health implications. Risk assessment methodologies should define standard targets or surrogates along with standard reporting methods (units) and agreed threshold levels.

Box 2: Potential measurement targets

Extended spectrum beta-lactamase (ESBL) *E. coli* provide a logical isolate-based target for AMR monitoring. Bacteria which produce these ESBL enzymes are often resistant to commonly used antibiotics.

Potential ARG , which have been suggested as useful targets for genotypic detection protocols, include:

- *intI1*
- *sul1, sul2*
- $bla_{CTX\text{-}M}$, bla_{TEM}, $bla_{NDM\text{-}1}$, bla_{OXA1}, bla_{VIM}, bla_{KPC}
- *qnrS*
- *ac-(6')-Ib-cr*
- *aph*
- *vanA*
- *mecA*
- *ermB, ermF*
- *tetM, tetW*

What other existing monitoring approaches could be adapted for AMR?

One existing monitoring approach for AMR is to use sentinel species as indicators, e.g. studying faecal samples from deer, birds and small mammals using traditional phenotypic/culture-based methods (Furness et al, 2017). Recent work has established baseline data across Scotland using AMR surveillance in wildlife hosts, particularly invertebrates, which are most relevant for water environments (Elsby, 2021).

Wastewater based epidemiology (WBE) has received much recent attention as an early warning system for a variety of waterborne infections. The strategy has been applied to monitoring community-wide drug abuse or chemical pollutants, and has also recently been applied to track SARS-CoV-2 levels in communities (Zahedi, 2021) and AMR (Sims, 2020; Pruden, 2021).

For AMR, levels of ARGs in wastewater can be measured, with the resistance profile in wastewater linking to the resistance profile of the population contributing to that wastewater. An exhaustive global review on WBE AMR surveillance highlighted that AMR characterisation in wastewater reflected AMR in human populations (Chau et al, 2021). As shown by a recent CREW report, the benefits of WBE AMR are the ability to undertake population-wide surveillance as well as the ability to track the impact of community-level interventions (Sims, 2021). Immediate implementation of AMR surveillance in wastewaters has been proposed (Pruden et al, 2021). However, in order to undertake WBE AMR surveillance, it is essential to determine standard targets (surrogates) and agreed threshold levels along with standard reporting methods to support risk assessment (Nyugen et al, 2021).

Find out more: For an overview of wastewater based epidemiology as applied to AMR monitoring, see the section of the full literature review entitled "Wastewater based epidemiology" (Appendix B)

What are the emerging methods of detection for antimicrobial resistance?

"Lab-on-a-chip" is an emerging technology with increasing popularity aimed to produce portable, user-friendly devices more easily used in field settings, which has been accelerated recently with the development of rapid COVID testing. The term refers to the miniaturisation of lab protocols towards portable cartridges integrating and automating multiple process steps. The term microfluidics is also used to describe the flow of samples through such devices. Integrated systems coupling sample handling and detection protocols offer advantages in testing. A variety of microfluidic tools have been developed, usually taking a phenotypic (bacteria growth-based) approach for medical applications. Several research publications report simple, robust and cost-effective lab-on-a-chip AMR testing, though this has yet to be fully exploited in the clinical setting, much less in the environment (Kaprou, 2021).

Sensor technologies can detect changes in bacterial growth or presence or amount of ARGs to report on AMR (Reali, 2019). Sensors can be divided into different categories based on the method of signal read-out, e.g. magnetic, mechanical, mass-based, electrochemical or optical. All types of sensors have been investigated for phenotypic susceptibility assays and performance tables can be found in the linked literature review. Genotypic methods have also been integrated into sensor platforms, using short sequences of nucleic acids to recognise and capture target genes. The most common types of sensor approaches are electrochemical and surface plasmon resonance (SPR), an optical method, and some approaches enabled detection of ARGs without prior amplification of the targets. However, the variation in reporting units makes it challenging to compare sensor performances. Additionally, there are still many challenges to be overcome to enable more widespread application of sensor technologies, which could be met by advances in materials, coupling with microfluidics and moving towards standardisation.

Gene sequencing is becoming more accessible and cheaper with the commercialisation of technologies such as Oxford MinION, or PacBio©. These methods offer long read sequences, that enable the presence of ARGs to be understood in the context of neighbouring genes. This gives valuable insight into the transmission and development of AMR along with the risks to human health. Computational technologies (bioinformatics pipelines) and new databases to analyse the resulting data are also emerging, further simplifying this approach to detection. There are research reports using the MinION to analyse clinical blood samples as well as environmental samples. This approach is likely to be very useful in screening for emerging ARGs.

In order to facilitate translation of emerging technologies to environmental AMR monitoring there are two key actions:

1. Determine existing current per sample cost of lab-based testing and present scenarios/use cases for surveillance approaches – define the economic landscape to enable technology developers to understand the commercial viability of different methods

2. Develop a Code of Practice for technology developers, incorporating economic and use case information with a list of key measurement considerations and a set of standards (e.g. genes and matrices) to enable validation of emerging tests and technologies. Important criteria for monitoring technologies for AMR in the environment are low-cost and ease-of-use along with an approach which offer consistent and reliable data. Multiplexing capability, to detect multiple targets within one test, is also desirable.

Dissemination of a Code of Practice should raise awareness of AMR in water as a monitoring challenge and drive further development. Organisations, such as CENSIS could promote this to research and commercial entities around Scotland to promote innovation and action on environmental AMR monitoring.

Find out more : For more details on the different types of emerging technologies and their application to AMR monitoring see the section of the full literature review entitled "Emerging Monitoring Technologies" (Appendix C)

What are the technologies for removal of AMR from water?

While wastewater treatment processes have not been specifically designed for AMR removal, wastewater treatment plants (WWTP) can effectively reduce total number of bacteria and the absolute abundance of ARGs (although the relative ARG abundance depends on the different genes). This was confirmed by many scientific studies, and feedback from expert questionnaires. However, factors such as pH and temperature within wastewater treatment plants influence their removal efficiency and little is known about the performance of individual stages in wastewater treatment processes for AMR (Hiller, 2019).

Methods for the removal of AMR from water can be divided into biological, physical and chemical approaches (see Box 3 and Table A1 in the Appendix A).

Box 3: Methods for wastewater treatment

BIOLOGICAL

- Conventional activated sludge (use of microorganisms to remove wastewater contaminants)
- Constructed wetlands (CW)
- Anaerobic membrane bioreactors (including developments to try to overcome fouling such as fluidised systems or those incorporating electrochemistry)

PHYSICAL

- Filtration methods (from traditional sand-based filters to membrane filters)
- Adsorption, e.g. to biochar

CHEMICAL

- Disinfection
- Oxidation processes

Constructed wetlands (CW) are also used in wastewater treatment and recent studies have reported up to 99% removal of ARGs, although, like WWTP, CW can also act as a reservoir for ARGs and a site of dissemination and transfer. More emerging technologies include:

- use of algae-based treatment, which at the lab scale has been shown to deactivate certain ARG plasmids
- employing new materials, either natural (e.g. fruit waste) or engineered, i.e. designed nanoparticles (e.g. zinc oxide or titanium dioxide) in disinfection processes
- phage-based therapy or graphene-based materials in adsorption processes

However, optimisation of wastewater treatment processes is not the only point to target to reduce AMR in waters. Reduction of faecal matter release into water should reduce downstream AMR. Additionally, source control of other contaminants (e.g. antibiotics, pharmaceuticals, personal care products, biocides, pesticides, heavy metals, nanoparticles, textile dyes, etc) which drive AMR is important and can be implemented at point of use. A cost-benefit analysis is required to determine the most effective approaches and to compare the costs, including carbon footprint, with the benefits against alternative strategies to control AMR.

One consideration for treatment technologies is the impact on the environmental microbiome. As microorganisms are the basis of the food web, removal of excess bacteria could damage biodiversity so treatment approaches should be effectively targeted at resistant bacteria. However, at present we lack an in-depth understanding of the potential impact and risks.

Find out more: For more information on treatment approaches to reduce AMR in water see the section of the full literature review entitled "Treatment Technologies" (Appendix D)

Methodologies/Limitations

A literature review focused on publications of the last five years (2017-2022) was undertaken, complemented with questionnaires to national and international experts and a workshop. The workshop participants spanned a range of academic disciplines (e.g. microbiology, water quality, AMR from both clinical and environmental perspectives, sensor technology, etc), industry, technology and innovation and policy. This analysis concentrated on AMR in bacteria, since the majority of the identified literature was in this area. However, further work should consider the impact of resistant viruses and fungi.

Policy Recommendations

Overall, there is no consensus on the best approach to monitor for AMR in water. Emerging technologies have adapted a range of existing methods to develop user-friendly, low-cost solutions, although there are still multiple issues to overcome before such approaches can be widely adopted. The knowledge gaps in relation to monitoring targets, baseline measurements and risk assessment approaches limit optimisation of technological solutions.

While there are developments in treatment technologies there is still a lack of knowledge relating to parameters influencing the performance of existing water treatment processes. Improving understanding of existing techniques is important. Additionally, a comparison of mitigation strategies should determine the most cost-effective approach to treat/reduce AMR in water.

Therefore, the recommendations to policy from this literature review and expert consultations are:

1. Create more opportunities for cross-sector networking and focused discussions. Find synergies between different sectors to drive monitoring and advancement of new technologies.
2. Establish a Scotland-wide baseline by incorporating AMR testing into existing sampling regimes.
3. Complement testing within existing sampling regimes with targeted AMR studies to address knowledge gaps.
4. Develop a Code of Practice to enable technology developers to understand different use cases, key measurement parameters, and validation methods.
5. Determine risk assessment parameters, enabling a link between monitoring data and actionable responses. There is a need for standard targets, standard reporting units and agreed threshold levels.
6. Undertake cost-benefit analysis of different treatment technologies and mitigation strategies. To support this analysis, there is a need to understand more about the impact of different strategies on the environmental microbiome and AMR persistence and transmission.
7. Raise awareness of the important role of water in AMR transmission and development, and communicate how responsible use of antibiotics can support decreased AMR in the environment.

References

Chau, K. et al, 2020, Systematic Review of Wastewater Surveillance of Antimicrobial Resistance in Human Populations. *Preprints* 2020100267 (doi: 10.20944/preprints202010.0267.v2).

Elsby DT. 2021. *Using wildlife to biomonitor for antimicrobial resistance in the Scottish environment* University of the Highlands and Islands

Furness, L.E. et al, 2017, Wild small mammals as sentinels for the environmental transmission of antimicrobial resistance, Environmental Research, 154, 28-34

Hiller, C.X. et al, 2019, Antibiotic microbial resistance (AMR) removal efficiencies by conventional and advanced wastewater treatment processes: A review, Science of The Total Environment,685,596-608,

Kaprou, G. et al, 2021, Rapid Methods for Antimicrobial Resistance Diagnostics, Antibiotics, 10, 2, 209

Nyugen, A.Q. et al, 2021, Monitoring antibiotic resistance genes in wastewater treatment: current 2 strategies and future challenges, Science of the Total Environment, 783, 146964

Pruden, A. et al, 2021, Seizing the moment: now is the time for integrated global surveillance of antimicrobial resistance in wastewater environments, Current Opinion in Microbiology, 64, 91-99

Reali, S. et al, 2019, Novel diagnostics for point-of-care bacterial detection and identification, RSC Advances, 9, 21486-21497

Sims, N. & Kasprzyk-Hordern, B., 2020, Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level, Environment International,139,105689

Sims N, Avery, L. and Kasprzyk-Hordern, B. 2021. Review of wastewater monitoring applications for public health and novel aspects of environmental quality (CD2020_07). Scotland's Centre of Expertise for Waters (CREW). Available online at: crew.ac.uk/publications

United Nations Environment Programme (2022). Environmental Dimensions of Antimicrobial Resistance: Summary for Policymakers.

Zahedi, A., Monis, P., Deere, D. *et al.* 2021, Wastewater-based epidemiology—surveillance and early detection of waterborne pathogens with a focus on SARS-CoV-2, *Cryptosporidium* and *Giardia*. *Parasitol Res* **120,** 4167–4188

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