CENTRE OF EXPERTISE FOR WATERS

Policy Note

Antimicrobial resistance in Scotland's waters

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Overview

- There is no baseline knowledge of the status of AMR in Scotland's Waters.
- Diverse detection methods could be complemented by some consistent approaches (e.g. testing of isolates for Extended Spectrum Beta-lactamase production (ESBL) – ESBL confers resistance to antibiotics such as penicillins and cephalosporins - and agreement of key resistance genes to target through molecular methods) to provide comparable reporting
- Expanding existing monitoring schemes to include AMR is key to understanding and mitigating this issue.

Over 700,000 deaths each year are due to diseases caused by drug resistant pathogens and failure to curtail antimicrobial resistance (AMR) could cause 10 million deaths per year globally by 2050 (WHO 2019). 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 (Box 1) to tackling AMR. We know little about AMR in Scotland's Waters and this present note addresses current understanding of the issue and the detection techniques and monitoring approaches that could be applied to generate a baseline understanding of AMR in Scotland's Waters.

BACKGROUND

It is increasingly recognised that antimicrobial resistance (AMR) is a major challenge facing society today. AMR in the environment is complex and the role of water in the proliferation and transmission of AMR is not well-understood. Here, we define the "water environment" as water bodies such as rivers & tributaries, lochs, groundwater, bathing and recreational waters, aquaculture, drinking water (source and tap) and wastewater.

We define antimicrobial resistance in its broadest sense, including antibacterial, antifungal, antiparasitic and antiviral resistances. Correlations between drivers of AMR (e.g., heavy metals, pharmaceuticals etc. released into water) and the dissemination and transfer of AMR in water have been established, but mechanisms of AMR development, proliferation, dissemination and transmission through water are poorly understood.

A baseline understanding of the current status of AMR in waters is important to proactively identify where and how to tackle this issue and to determine the success of mitigation approaches going forward.

AMR in waters is part of the UK 20-year Vision for Antimicrobial Resistance, the *UK 5-year National Action Plan for Antimicrobial Resistance* (UK NAP) 2019-2024, and for the *Scottish One Health National AMR Action Plan* (SOHNAP) group, which feeds into the UK NAP. By adopting a 'One-Health' approach to tackling AMR, there is appropriate emphasis on the importance of the environment in the transmission of AMR. The topic is also pertinent to the River Basin Management Planning 2021-27 and Scottish River Basin District (Standards) Directions 2014 and subsequent amendments, Bathing Waters Scotland Regulations (2008) and to the Water Environment (Shellfish Water Protected Areas: Environmental Objectives etc.) [Scotland] Regulations 2013. It is important to address AMR across the different policy areas pertinent to water, the environment and human, animal and plant health (United Nations Environment Programme 2022)

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Box 1: ONE-HEALTH DEFINITION

"One Health is an **integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems**. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent" – (One Health Commission 2021).

We used literature evidence and elicited the opinion of academic experts and key stakeholders through questionnaires and a workshop to better understand the status of AMR in Scotland's waters, and the role of technologies (see associated Policy Brief by Bridle et al., 2022) and monitoring approaches for AMR surveillance. Key findings are highlighted.

CURRENT STATUS OF AMR IN SCOTLAND'S WATERS

We identified few published/publicly available studies pertaining to AMR in waters in Scotland. Those found included three studies on tap (drinking) water. In the first study Khan et al. (2016b), found that resistance to sulphonamides and markers for transferable genetic elements were detected across a range of different bacterial genera. In the second study, the authors noted a weak correlation between chlorine tolerance and antimicrobial susceptibility (Khan et al 2016a). The third study demonstrated selection for resistance under sub-lethal concentrations of chlorinated disinfectants (Khan et al 2017).

Two studies related to estuarine sediments: Tonner et al., (2019) assessed the susceptibility of bacterial isolates from the Inner Clyde estuary to antibiotics and metals, AMR being greater where isolates originated from sites with greater metal pollution. Rodgers et al. (2020) did not measure AMR but did identify sediment hotspots for metals in the Clyde estuary.

Authors of a study on the impact of anthropogenic activity on AMR in wild birds Djuwanto (2021) found ESBL producing *E. coli* in 57% of the faeces samples of seagulls (n=47) sampled at an Edinburgh wastewater treatment plant (WWTP) compared with only 2% of the faeces of seagulls (n=50) sampled at a coastal location (Berwickshire, SE Scotland).

In a PhD study on sentinel species for AMR monitoring, Elsby (2021) isolated resistant *E. coli* from the guts of freshwater invertebrates at five river sites on the rivers Naver, Thurso and Wick, fortnightly over 16 weeks during spring/summer. Antecedent rainfall significantly influenced both non-resistant and resistant *E. coli*.

In a study of *Flavobacterium psychrophilum* isolates from trout or salmon, where the majority of isolates were from Scotland, 88% of UK strains were resistant to oxolinic acid and 58% to oxytetracycline (Ngo et al 2018).

AMR Monitoring was built into bathing water sampling activities in 2018 (SEPA 2022). *E. coli* isolates are tested for cefotaxime resistance. Vancomycin resistant enterococci will also be monitored from 2022. This provides arguably the most comprehensive publicly available data on AMR in waters in Scotland.

A number of datasets are currently being prepared for academic publication e.g. reporting on a comprehensive suite of resistance genes across a range of Scotland's rivers (Avery 2022a, Avery 2022b, Pagaling 2022a, Pagaling 2022b)

In conclusion, to date, there is no comprehensive baseline understanding of the current status of AMR in Scotland's water bodies, with no formal monitoring for resistance in in-land waters.

CURRENT DETECTION METHODS AND BIASES

Different AMR detection methods are employed across research and monitoring efforts. Diverse approaches are useful for research. However, inconsistencies lead to difficulty in comparing across studies and across sectors within a One-Health approach.

Most studies measured either antibiotic resistance genes (ARGs) or antibiotic susceptibility of bacteria isolated from the environment. Metagenomics and whole genome sequencing were also prominent approaches. There was no consensus regarding the best detection technique to use.

Methods highlighted by experts through our workshop and questionnaires included PCR-based techniques (40%) and cultivation and susceptibility testing (30%), metagenomic sequencing (15%), whole genome sequencing (5%), Raman spectroscopy, chromatography, lateral flow test and flow cytometry (2.5% each). Cultivation-based techniques are relatively straightforward and inexpensive, and are therefore more accessible. The more complex and expensive methods (e.g., metagenomics) are limited to those with access to bioinformatics expertise and larger sources of funding. Participants largely agreed that the choice of detection method depends on the monitoring purpose (Box 2) and strategy. There is not a 'one size fits all' detection method. A 'toolbox' approach was suggested to allow researchers more flexibility. However, **we recommend agreeing and incorporating some minimum common approaches to allow studies to be comparable**. For example, for cultivation-based approaches, ESBL *E. coli* could be measured to allow results to be compared e.g. to those obtained through the WHO tricycle scheme, and for PCR-based methods, there could be a suite of selected resistance genes that should be measured.

Consistency in the chosen methods can be further developed through consideration of sampling (volumes, handling, storage), protocols (SOPs, researcher training), and data processing (analysis, interpretation, visualisation, management, storage, quality assurance and sharing). **Further workshops/working groups are needed to agree on standard procedures and the common ARG targets that should be measured.**

MONITORING STRATEGIES

It is important that we do undertake monitoring of Scotland's waters to develop our understanding of the environmental dimension of AMR and to provide a current status baseline which does not currently exist. The findings of our questionnaire study and workshop tied in with the literature e.g. (Hayhurst 2021, Pruden 2018) regarding the questions as to what, where and how to monitor.

Ahead of deciding what to monitor, **it is important to define the purpose of measuring AMR in waters (or wider environment). Who/what do we want to protect? Who is going to use the data?** (Box 2).

Box 2: Purpose of Monitoring

Address risk of:

- Transmission of an existing antibiotic resistant (AR) pathogen via environmental routes
- Accelerating evolution of new AR pathogens through pollution by selective agents, ARGs and bacteria of human or animal origin
- Antibiotics to ecosystem health

To monitor amongst the population:

- Prevalence of resistance
- Consumption amongst population

(Modified from Hayhurst et al., 2021 and Huijbers, Flach et al., 2019)

The main driver for addressing AMR is to prevent resistant (i.e., difficult to treat) infections in humans and animals. This, therefore, requires an ability to link measurements in the environment with clinical or veterinary outcomes. AMR could also impact the wider ecological health and quality of water bodies. To understand these ecological processes and evolution of AMR in rivers, we may need to measure something that provides a "bigger picture" assessment of AMR. Because questions remain regarding exactly where and how new resistances are likely to develop, overview surveys e.g. of resistance genes, may facilitate the detection of emerging threats. **A significant knowledge gap is how ARGs or antibiotic resistance bacteria (ARB) in the environment translate into ARBs in clinical settings**. Describing the sources, pathways and receptors in a risk-based framework would allow environmental data to be interpreted in line with these objectives. This could be facilitated through a DPSIR (Drivers, Pressures, State, Impact, Response) approach similar to that underpinning the Water Framework Directive.

Box 3: What to Monitor:

- WWTPs (effluent and/or influent)
- Combined Sewer Overflows
- SuDS
- Industrial discharges
- Drinking water (source or tap)
- Rivers/ receiving waters (including septic tank/care home receiving waters)
- Bathing or recreational waters (whether designated or not)
- Aquaculture settings
- Agricultural environment including water-courses impacted by farms
- Animals and Humans
- Soils, sediment, biofilm
- Biosolids

WHERE SHOULD WE BE MONITORING AMR?

While not unique to Scotland, there are aspects of Scotland's water environment that may influence the nature or locations where monitoring is deemed important. For example, the extensive rural land area necessitates a substantial number of private drinking water supplies and septic tanks. Scotland is known for its fisheries and aquaculture industry. Catchments vary in character from highland to lowland, urban to rural and arable to livestock dominated. Furthermore, the natural environment is host to numerous non-designated bathing waters used for bathing and recreational activities. Literature and expert opinion indicated a need to monitor a range of water-related sources, pathways and receptors (Box 3).

A cross-cutting, interdisciplinary approach, encompassing "One-Health" (human, livestock, environment) goals and taking note of the economic and social landscapes is important for any monitoring approaches taken forward (Box 4). Furthermore, AMR is an environment-wide issue, and aspects such as aerosol transmission and application of organic wastes to land could play a key part in transmission sources and pathways. Thus, methodological approaches must be suited to specific sectors. For example, spatio-temporal approaches generating a large number of samples may be needed to adequately characterise a flowing water body. In this case, storage of water filters for subsequent high throughput qPCR may be a more practical way of gaining an overview of AMR in waters, whereas application of isolate-based methods (requiring immediate full processing of the samples) presents logistical difficulties.

It is widely accepted that alongside the presence of antimicrobials in water, other chemicals act as co-selectors – compounds which can induce cross-resistance against antimicrobials. There are still may unknowns about their relationship with AMR (Box 4 and related CREW Policy Brief by Bridle et al., 2022). **To this end, pharmaceuticals, co-selectors, standard water quality parameters and ecological determinants (e.g., water microbiome, invertebrates) should be measured alongside determinants of resistance, where possible (see Recommendations in CREW Policy Brief by Alejandre et al. 2022)**. Reducing faecal inputs from both human and animal sources is expected to reduce AMR, faecal bacteria and pathogens and nutrients (as well as other chemical pollutants) and assessing proxies for AMR detection is worthy of further investigation.

Building on existing sampling regimes already being undertaken (e.g. regulatory monitoring of bathing waters, shellfish, drinking water sources and wastewater) is likely to be the most cost-effective approach to monitor AMR in waters. However, this may not fulfil surveillance of all water typologies of interest (e.g., rivers and lochs not impacted by WWTP) and may therefore fall short of enabling a full picture of AMR in Scotland's Waters. **Therefore, we would recommend focussing on existing sampling regimes plus additional selected river and loch monitoring campaigns**. The novel platforms introduced for influent- sewage-based monitoring of SARS-CoV-2 could be exploited to monitor resistance at population level and, coupled with WWTP effluent monitoring, entering the environment. This would facilitate the prediction of changing patterns in both disease and resistance genes (Sims 2021).

Box 4: Ideally, monitoring needs to capture:

- Variation: seasonal, diurnal, rainfall
- Sources & impacts: including industrial pollution, waste management & application
- Awareness or tracking of pollutant sources
- Awareness of polluter behaviours
- Up and downstream of sources
- Regularity & consistency

KEY RECOMMENDATIONS

1. Create more opportunities for cross-sector networking and focussed discussions. Build multidisciplinary, multi-actor forum for discussion of opportunities and approaches.
2. Measure pharmaceuticals and co-selectors concurrently with AMR targets.
3. Develop AMR monitoring guidelines with research and regulatory community to promote consistency across studies.
4. Build AMR monitoring into existing sampling regimes with additional targeted AMR studies to address knowledge gaps including i) establishing a baseline for AMR in Scotland's Waters and ii) understanding how ARGs or ARBs in the environment translate into health outcomes.

REFERENCES

Avery L, Pagaling, E., Bridle, H. et al. 2022a. River Ugie ARG dataset.

Avery L, Pagaling, E., Newman, C., Robinson, L., Cooper, P., et. al. 2022b. National Waters Inventory for Scotland

Bridle, H., Pagaling, E. and Avery, L. (2022). Technologies for Monitoring and Treatment of Antimicrobial Resistance in Water: CREW Policy Brief. CRW2020_19 CREW Policy Fellowships Programme. Scotland's Centre of Expertise for Waters (CREW). Available online at: https://www.crew.ac.uk/publications

Djuwanto B. 2021. *Wild Birds as a Reservoir of Antimicrobial Resistance (AMR) in the Environment*. Edinburgh Napier University

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

Hayhurst EaE, S. . 2021. Antimicrobial Resistance Literature Review

Huijbers PMC, Flach C-F, Larsson DGJ. 2019. A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance. Environment international 130: 104880

Khan S, Beattie TK, Knapp CW. 2016a. Relationship between antibiotic- and disinfectant-resistance profiles in bacteria harvested from tap water. *Chemosphere* 152: 132-41

Khan S, Beattie TK, Knapp CW. 2017. The use of minimum selectable concentrations (MSCs) for determining the selection of antimicrobial resistant bacteria. *Ecotoxicology* 26: 283-92

Khan S, Knapp CW, Beattie TK. 2016b. Antibiotic Resistant Bacteria Found in Municipal Drinking Water. *Environmental Processes* 3: 541-52

Ngo TPH, Smith P, Bartie KL, Thompson KD, Verner-Jeffreys DW, et al. 2018. Antimicrobial susceptibility of Flavobacterium psychrophilum isolates from the United Kingdom. *Journal of Fish Diseases* 41: 309-20

One Health Commission. 2021.

Pagaling EaG, M. 2022a. Cessnock Water and Mein Water AMR Dataset. ed. JH Insitute

Pagaling P, Stutter, M. ,Avery, L. et al.,. 2022b. River Dee AMR sampling.

Pruden A, Alcalde, R., Pedro., J.J. et al. 2018. An Environmental Science and Engineering Framework for Combating Antimicrobial Resistance. *Environmental Engineering Science* 35: 1005-11

Rodgers K, McLellan I, Peshkur T, Williams R, Tonner R, et al. 2020. The legacy of industrial pollution in estuarine sediments: spatial and temporal variability implications for ecosystem stress. *Environmental Geochemistry and Health* 42: 1057-68

SEPA. 2022. AMR Monitoring.

Sims N, Avery, L.isa and Kasprzyk-Hordern, Barbara,,. 2021. Review of wastewater monitoring applications for public health and novel aspects of environmental quality, Scotland's Centre of Expertise for Waters (CREW).

Tonner R, Peshkur T, Rodgers K, MacLellan I, Williams R, et al. 2019. Co-selection of antibiotic resistance caused by a legacy of PTE pollution in Gram-negative bacteria. *Access Microbiology* 1

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

WHO. 2019. Joint News Release. World Health Organisation

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