Environment and Rural Affairs Monitoring & Modelling Programme (ERAMMP)

ERAMMP Report-55: Evidence Review on the Entry and Spread of Antimicrobial Resistance (AMR) in the Rural Water Environment in Wales

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Abbreviations Used in this Report

AMR	Antimicrobial resistance
ARB	Antimicrobial resistant bacteria
ARDC	Antimicrobial resistance driving chemicals
ARG	Antimicrobial resistance genes
CSO	Combined sewer/sewage overflows
CTX-M	CTX-M beta-lactamases (derived from cefotaxime [CTX as its acronym], and -M from Munich)
DCWW	Dŵr Cymru Welsh Water
DNA	Deoxyribonucleic acid
ERAMMP	Environment and Rural Affairs Monitoring & Modelling Programme
ESBL	Extended-spectrum beta-lactamase
GP	General practitioners [primary care doctors]
HGT	Horizontal gene transfer
intI1	*intI1* (an integron-integrase gene encoding for part of a class 1 integron which acts as a mobile genetic element that is operationally used as a proxy for sewage-polluted water, and often co-correlates with elevated antimicrobial resistance)
IPR	Indirect potable reuse
MCSC	Minimum co-selective concentration
MGE	Mobile genetic elements
MIC	Minimum inhibitory concentration
MSC	Minimum selective concentration
ng/L	Nanograms per litre
PCR	Polymerase chain reaction (PCR)
PPE	Personal protective equipment
QACs	Quaternary ammonium compounds
qPCR	Quantitative PCR
SELECT	Selection end-points in communities of bacteria
SUD	Sustainable Urban Drainage
ug/L	Micrograms per litre
UKCEH	UK Centre for Ecology & Hydrology
UV	Ultraviolet
WG	Welsh Government
WWTP	Wastewater treatment plant

Abbreviations and some of the technical terms used in this report are expanded on in the programme glossaries: https://erammp.wales/en/glossary (English) and https://erammp.cymru/geirfa (Welsh)

Contents

1 Executive Summary

Antimicrobial resistance (AMR) refers to the capacity of microorganisms to resist the effects of otherwise inhibitory chemicals - an attribute that can be intrinsic or acquired.

Acquired AMR is at the core of the global increase in drug-resistant infections, representing a healthcare emergency, costing the NHS an estimated £30 billion per year to treat infections and infectious diseases in England [1]. Mobile genetic elements (MGEs) facilitate the exchange of antimicrobial resistance genes (ARGs) within and between microorganisms, enabling their rapid and global dissemination.

Antimicrobials (antivirals, antibacterials, antifungals, antiprotozoals, anthelmintics) represent a fraction of all chemicals that are known to select for or aid in the dispersal of ARGs. The chemicals that drive AMR, i.e., antimicrobial resistance-driving chemicals (ARDCs), include metals, biocides, pesticides and many other environmental pollutants.

Areas of significant anthropogenic impact can be areas of elevated AMR, including manufacturing and industry discharge, agriculture, municipal wastewater (wastewater treatment plants [WWTPs], combined-sewer outfalls, sewage sludge) and meat animal, egg, sport animal and dairy production activities (feed, chemotherapy, biosecurity, manure, slurry).

Concentrations of ARDCs that do not kill bacteria (i.e. sub-lethal concentrations) have been shown to be sufficient to drive the selection and mobilisation of ARGs in many environmental compartments, e.g., freshwater and soils, as would also be the case in humans, e.g., the gut. In addition to chemical pollution, environmental pollutants that can increase the dissemination of ARGs represent a major mechanism for ARG dissemination, particularly from major human and animal waste sources such as wastewater and meat animal production.

Source reduction of ARDCs and ARGs is likely the most important mitigating measure to reduce the hazard from AMR in the environment. Source reduction can be achieved by tackling the **direct drivers**, namely:

1. Improving wastewater treatment to reduce the discharge of ARDCs and ARGs into the environment.
2. Improving the treatment of sewage sludge and manure through anaerobic digestion and stabilisation technologies like pyrolysis (heat-induced decomposition of organic materials).
3. Measure, report and reduce antimicrobial and ARDC use in meat animal, egg, sport animal and dairy production activities, particularly metals, which impose a co-selection hazard for AMR in manure and impose a burden on receiving soils.
4. Improve monitoring of ARDC and ARG burdens in the environment, and better regulation of environmental pollution, globally.
5. Eliminating the need for combined sewer overflows and storm overflows which represent a short-circuit in the treatment pathway for ARDC-rich wastewater. Eliminating sewer and storm overflows can be achieved by: a) expanding sewage network capacity, addressing population increase and heavier and more frequent precipitation; b) implementing sustainable urban drainage (retrospective and into the future), and reducing water use per capita.

Source reduction can also be achieved by tackling **indirect drivers**:

1. Reduce human and animal infections through greater reliance on: new and existing vaccinations, improved infection prevention and control, and better animal welfare.
2. Reduce antimicrobial use through improved pathogen diagnostics, rapid determination of antimicrobial susceptibility and better antimicrobial stewardship in human and veterinary medicine.

The environmental dimension of AMR in Wales is poorly characterised at present.

A surveillance campaign of direct and indirect drivers will help to establish a baseline for quantifying the success of future mitigation measures. Developing a strategy for tackling the environmental dimension of AMR will necessitate an understanding of the impact of climate change (i.e., dryer summers and wetter winters, with heavier periods of rain), changes and increases in meat animal and animal product production, and human population growth on the environmental dimension of AMR, in Wales.

2 Introduction

The Welsh Government (WG) commission for this review, within ERAMMP, is to identify how AMR:

- "enters and spreads via the rural water environment"
- "impacts on animal and human health"
- can be "tackled at Welsh-level using an integrated policy approach to ensure the effectiveness of antibiotics for future generations."

This Evidence Review is intended to inform policy on the delivery of the Welsh Government's 5-year 'Antimicrobial resistance in animals and the environment implementation plan'. Specifically, it will inform the third component – Minimising the spread of AMR through the environment, with a focus on water sources. This component commits to support research into identifying evidence gaps and improving understanding of the hazards and risks from AMR in the environment.

The review team were asked to identify options for follow-up research and possible policy directions, compatible with the wider legislation framework, notably the Well-being of Future Generations (Wales) Act 2015 and Environment (Wales) Act 2016 and associated UN Sustainable Development Goals.

3 Fundamental Principles of the Environmental Dimension of AMR

3.1 Introduction to AMR

Current estimates are that 700,000 people die each year worldwide from antimicrobial-resistant infections, and it is predicted that if action is not taken that number could reach 10 million people and cost an estimated US$100 trillion by 2050[2]. As a result, the clinical emergence of AMR in human and animal pathogens is recognised as one of the major Global Health Challenges of the 21st century[3].

AMR refers to the capacity for microorganisms to resist the toxic or otherwise inhibitory effects of chemicals in their environment. Antimicrobials are agents that kill or stop the growth of microorganisms; the term refers to a broad suite of chemicals with a wide range of targets, inclusive of:

- antivirals that target viruses
- antibiotics that target bacteria
- antifungals that target fungi
- antiprotozoals that target protists
- anthelmintics that target helminths/worms

The growing trend of AMR in clinically-relevant pathogens directly results from the widespread use and misuse of antimicrobials, not just in the clinical setting, but throughout society, for example, human healthcare, meat, dairy, and egg production, aquaculture, fruit production, food preservation, cereal crop agriculture and paint preservatives.

The legacy of industrial activities can also serve as an important source of chemicals that can contribute to AMR, such as metals from mining[4] or war zones/(practice) battlefields[5], as well as the natural geological variability in metal availability within soils[6]. Most antimicrobials persist in vivo, which results in the excretion of at least 50% of the parent compound in urine and faeces. Human antimicrobial use drives wastewater concentrations and concentrations found in the downstream, aquatic environment[7].

A new holistic perspective considering AMR drivers from human, animal, and agricultural use and pollution in the environment is at the core of the "One Health" approach. This evidence review will be primarily focused on the environmental dimension of AMR, which is directly and indirectly impacted by all segments of society. The literature based on the environmental dimension of AMR is primarily focused on antibacterial resistance – a bias that is reflected in this review.

3.2 Intrinsic and Acquired Antimicrobial Resistance

Bacteria and fungi can resist antimicrobials through two main mechanisms**: intrinsic resistance** and **acquired resistance**. Intrinsic resistance is achieved through naturally-occurring resistance mechanisms (e.g., an impermeable cell wall or chromosomally-encoded efflux pumps) that are passed on vertically, i.e., from mother cell to daughter cell, and are largely fixed attributes in specific bacterial species. Intrinsic resistance mechanisms pre-date antibiotic chemotherapy[8] by (likely) many millions of years[9]. Although intrinsic resistance to antimicrobials has clinical relevance[10], it is acquired resistance that is at the heart of the current global AMR crisis.

Acquired resistance is the capacity of a microorganism to become resistant to an antimicrobial to which it was previously susceptible. This occurs as a result of DNA mutagenesis (point mutations, deletions or duplications) or acquisition of ARGs from other bacteria through horizontal gene transfer (HGT). Acquired resistance mechanisms are genetically and phenotypically highly varied, and their impact depends upon the microorganism carrying them and the presence of other co-located ARGs, whether they confer resistance to clinically relevant antimicrobials, and surrounding environmental factors that can affect how they are expressed. Evolution or acquisition of MGEs that contain ARGs are two common mechanisms of acquired resistance.

A wide range of mechanisms of acquired resistance have been documented[11], namely:

- Permeability changes in the bacterial cell wall that restrict antimicrobial access to target sites
- Active removal of the antimicrobial from the microbial cell via acquired efflux pumps
- Enzymatic modification of the antimicrobial
- Degradation of the antimicrobial agent
- Acquisition of alternative metabolic pathways to those inhibited by the antimicrobial
- Modification of antimicrobial targets
- Overproduction of the target enzyme for the antimicrobial

Acquired resistance has been reported from the earliest use of sulfamethylthiazole in the 1930s[12]. Evidence for the evolution of antibiotic resistance has been documented for every antibiotic shortly after its introduction[13]. However, the emergence, selection, mutation and persistence of novel ARGs in a population will depend on the fitness cost, i.e., the net cost-benefit to the microorganism for carrying the new resistance trait[14].

An analogy is provided to illustrate this point: traditional wheeled vehicles were fitted with tank treads to allow navigation over all kinds of terrain with increased stability, traction and torque, but the acquisition of tank treads introduces a ‘fitness cost’ of high operating energy, slow speed, reduced finesse, and increased maintenance. Unlike vehicles with tank treads, microorganisms can sometimes evolve ways to compensate for unfavourable cost-benefit ratios (e.g., epistasis and non-epistatic mechanisms)[14,15], thereby establishing the new resistance trait in the population and risking its global spread in different microbial hosts and environments[16].

It is also notable that some resistance mechanisms come with little to no fitness cost, for example, a particular type of streptomycin resistance carried by *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Escherichia coli*, which is caused by a mutation in the *rpsL* gene [17,18].

Mobile genetic elements (MGEs) are ancient replicating entities that drive the evolution of bacteria and fungi[19]; their importance has been highlighted in the last 80 years since the invention and widespread use of antimicrobials[20]. MGEs allow successful genomic solutions to the toxic effects of antimicrobials to be horizontally transferred from one microorganism to another. MGEs can be transferred through a range of genomic ‘hardware’, including: plasmids, gene cassettes, integrons, transposons, insertion sequences and bacteriophages[21]. Che et al. (2021) recently confirmed the importance of MGEs in HGT and in disseminating ARGs among distantly-related microorganisms within an ecosystem, as well as reshuffling ARGs within individual organisms and species[22].

In the specific case of bacteriophages, ARGs move from one bacteria to another through the accidental incorporation of DNA from the host bacterial cell within the bacteriophage and its successful transfer to the recipient bacterial cell upon infection. In much the same way that a pandemic is very unlikely to occur in the absence of the movement of people, a global AMR crisis would be much less likely if not for the mobility of ARGs—novel genes don’t cause pandemics, mobilisation and transmission does.

3.3 Selection, Dissemination and Transmission of AMR in the Environment

The global problem of drug-resistant infections can be understood through the lens of three key actions: selection, dissemination and transmission of AMR.

3.3.1 Selection

Selection for AMR is when a chemical compound or environmental influence results in the increase of the proportion of antimicrobial resistant bacteria in comparison to antimicrobial susceptible bacteria.

The hazard of AMR selection is maximised when microbial abundance, ARDC concentration and longer durations of exposure are all present[23]. Environments where these three elements are maximised typically include: wastewater, WWTPs, animal and human excreta, compost, and some aquaculture.

There are no objective metrics of what defines high microbial abundance, chemical concentration or duration of exposure – these parameters are most often defined relationally and situationally, and may be variable for different ARG-bacteria combinations. For example, a fish that defecates into an oligotrophic (i.e., low nutrient) water body will have introduced a high microbial waste material into a low nutrient environment. It might then be expected that the duration of release and the concentration of waste will be too low to impact AMR selection.

However, it would be very different if instead there were thousands of fish and they were confined to an aquaculture facility where they were fed antiparasitics, antibiotics and/or antifungals, all of which accumulated in a layer of fish faeces (containing antimicrobials and a high microbial load) under the aquaculture enclosure. Locations, where microbial abundance, chemical concentration, and exposure are maximised, are more likely to be places of AMR selection.

Other locations where AMR selection is likely to be elevated include outfalls for treated wastewater and combined sewer overflows (CSOs)[24], slurry lagoons containing animal waste[25] and industrial discharge points[26].

Chemical Concentration: Minimum inhibitory concentrations (MICs) are the lowest concentration of an antibiotic that will inhibit the visible growth of a microorganism and are routinely used in the clinical setting to establish phenotypic resistance and guide treatment options. The lowest concentration that offers an antimicrobial-resistant microorganism a selective advantage over its otherwise genomically-identical and antimicrobial-sensitive clone is called the minimum selective concentration (MSC).

There have been several studies that attempted to empirically determine the MSC for particular drug-bacteria combinations, but due to the abundance of antimicrobials and the high taxonomic diversity of microorganisms, it remains currently impractical to empirically generate such thresholds for all species or strains. However, single-cell and high-throughput automated screening are achievable in the foreseeable future. More recently, studies have focused on mixed microbial communities in an effort to explore the effect of antibiotic concentrations on more realistic environments,[27–30] but these studies used lengthy processes that require 7+ days to determine MSC values.

A study published in 2020 developed a novel method (SELECT method) that produces empirical data in 24 hours which would allow rapid testing of antibiotic compounds and other ARDCs[31]. Models have also been used to estimate MSCs from measured environmental concentrations and have indicated that MSCs could be up to two orders of magnitude lower than the MIC[32]. Empirical evidence often, but not always, corroborates such estimates[27–29,31,33,34].

Exposure Time: Chronic exposure of a microorganism to an antimicrobial is more likely to select for resistance. In practice, resistance can develop very quickly (Frimodt-Møller and Løbner-Olesen, 2019) [35] and spread within a community in the order of hours to days (Maddamsetti and Lenski, 2018)

[36]. Episodic antimicrobial exposure, i.e., released into the environment as a pulse, might be expected to have a different impact on microbial resistance selection than chronic exposure.

Examples of episodic exposure in the environment include the release of untreated wastewater from storming or CSOs during high rainfall events, which can last from minutes to days[24]. In these scenarios, the downstream freshwater environment is exposed to 10-1000 times higher bacterial and chemical loads than would be typical of a treated sewage outfall[37–39]. There is little in the literature to illustrate the AMR hazard such outfalls pose to humans, however Brokamp et al. (2017) reported an increased risk from gastrointestinal-related emergency department visits following CSO events among children who reside near CSO sites [40].

Antimicrobial-Resistance Driving Chemicals (ARDCs): The maintenance and spread of AMR in the environment is a multifaceted problem, which is not caused by one variable alone (e.g., a bacterial species exposed to an antibiotic), but rather a combination of events exposing complex communities to a range of chemicals and various environmental factors.

Antimicrobials are not the only chemical drivers of AMR (Table 1). The presence of (growth-)inhibiting concentrations of chemicals such as metals, biocides, pesticides, and herbicides have been shown to drive mechanisms of resistance, as well as natural factors such as soil pH and geology[6]. At times, resistance mechanisms fortuitously offer protection to one or more antimicrobial, by either individually resulting in resistance to several classes of drug, or by co-occurring with other ARGs in the same bacterial cell.

This phenomenon is generically called co-selection, which is the capacity for one class of chemical to select for resistance to another class of chemical. Co-selection has been demonstrated in a very wide range of chemicals, making it challenging to rule out the co-selective role of any chemical.

ARDCs that risk co-selection at low concentrations are most concerning. Metals and biocides are among the more well-studied co-selective agents in widespread global use. Metals are routinely used in clinical, industrial and household settings for their antibacterial properties (e.g., copper and silver), while they also constitute nutritional supplements in animal meat production, including aquaculture[41–43]. It has been proposed that the correlation of metals with ARGs, in some cases, may be even higher than that of antibiotics with ARGs (Ji et al. 2012)[44]. Metals can be regionally important freshwater pollutants where historical mining was once present, as is common in Wales[45], where industry has left a legacy[46,47], and in soils downwind of atmospheric point sources [48]

Specific metals, like iron and aluminium, can also be added as coagulants to wastewater to aid in the clarification of the waste stream[49]. Roads routinely receive and release a wide range of metals from tyres, catalytic converters and brake pads – all of which can run off into soakaways, when they are present, or the nearest watercourse[50]. As such, metals represent a significant ARDC hazard, particularly to the freshwater environment, which receives a mix of treated and untreated sewage, road and farm runoff, and mining and industrial pollution.

Biocides (e.g., disinfectants and surfactants) are routinely used in cleaning products in clinical, agricultural, industrial, and household settings, as well as in personal care products [7]. Biocides have been shown to select for AMR, for example, triclosan and quaternary ammonium compounds (QACs) [51,52].

Limited work has been undertaken to quantify the selective properties of co-selective agents and determine minimum co-selective concentrations (MCSCs) in complex microbial communities. However, MCSCs have previously been calculated for a range of metals using mathematical modelling [53] or using single species competition experiments [54].

The co-location of multiple ARDCs in many environments makes it impossible to conclusively identify the main chemical driver of AMR[55–57]. Such conditions are common and complicate the task of prioritising specific pollutants for mitigation.

Table 1 ARDCs reported in the literature, where ARDCs include chemicals that increase the HGT activity of MGEs.

Antimicrobial-Resistance Driving Chemicals	References
Metals (e.g., copper, zinc, arsenic, cadmium)	58,59
Biocides (e.g., Quaternary ammonium compounds: cetylpyridinium chloride, chlorine, benzalkonium chloride; chlorhexidine, and triclosan; disinfectants: chlorine, chloramine, hydrogen peroxide, chlorite, iodoacetic acid bromoacetamide, trichloroacetonitrile, tribromonitromethane; nanometals: nano-Al_2O_3, nano-TiO_2, nano-SiO_2, and nano-Fe_2O_3)	59–70
Herbicides (e.g., glyphosate, dicamba, 2,4-dichlorophenoxyacetic acid)	71,72
Non-Antimicrobial Pharmaceuticals (e.g., carbamazepine, ibuprofen, naproxen, diclofenac, gemfibrozil)	73
Other (e.g., saccharine, sucralose, aspartame and acesulfame potassium)	58

3.3.2 Dissemination

Dissemination is defined as the spread of antimicrobial resistant bacteria (ARB) and ARGs through different environmental niches.

In the environmental context, ARB are disseminated from ‘leaky’ locations of high anthropogenic impact, and ARGs are disseminated by ARB through HGT. Environmental systems are exposed to – and therefore, often the recipient of – these “leaky” sources, which include WWTP outfalls and CSOs, aquaculture, landfill and septic tank leachate, road runoff, industry liquid and atmospheric discharge points, and farming and agricultural runoffs, such as those from land application of sludge and animal waste, leaking slurry tanks, animal husbandry and crop treatments (Figure 3.1).

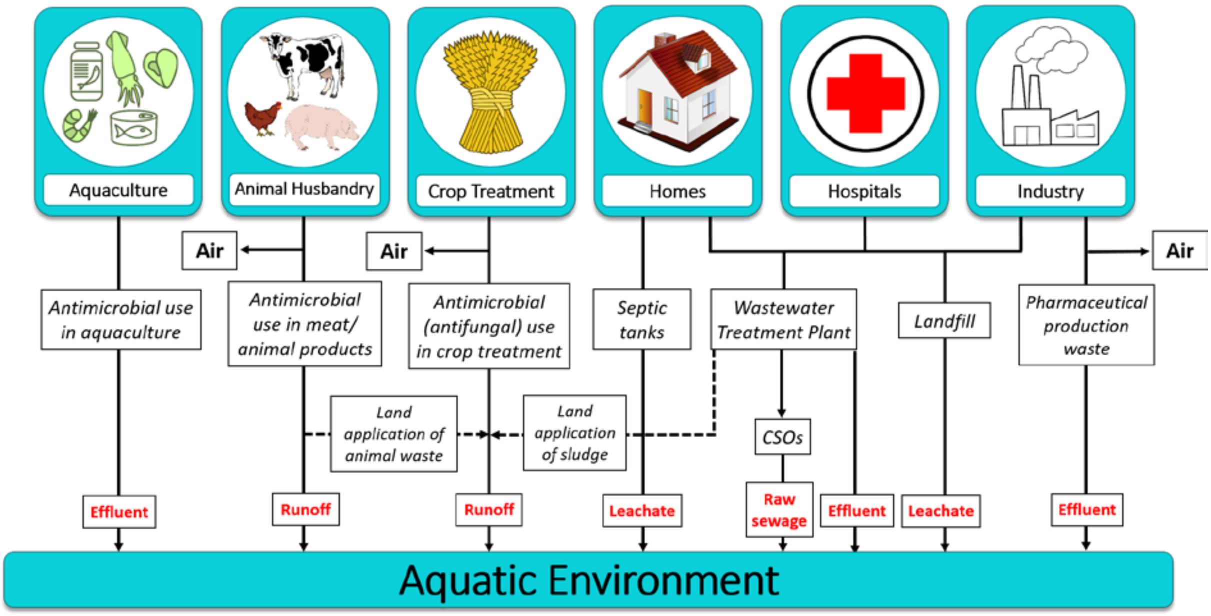

Figure 3.1 Environmental dissemination pathways for ARDCs, ARGs and ARBs

For example, in agricultural systems, slurry, sewage sludge and anaerobic digestate are used as fertiliser for crops, possibly disseminating ARB and ARGs to food crops, farmers, livestock, wildlife and environmental niches.

Evidence suggests that migratory birds are also able to disseminate ARB from one environment to another, for example, through their faeces [74,75]. Recent studies reveal that microorganisms are able to colonise the surfaces of microplastics and nanoplastic aggregates. Plastic serves as a vehicle for the effective dissemination of ARGs in the freshwater environment[76]. The high density of microorganisms and ARGs on plastic suspended in freshwater increases the theoretical risk of ARG ingestion and transmission to humans.

3.3.3 Transmission

Transmission is used here to describe the transmission of ARB and ARGs from the environment to humans.

Exposure to ARB and ARGs presents a public health risk. ARB can be of immediate clinical relevance (e.g., *Enterococcus faecium*[77], *Escherichia coli*[78]) or they can be microorganisms of no clinical importance, but harbour mobile ARGs that can transfer to clinically-relevant microorganisms within the hosts they colonise, e.g., in the gut[79].

Transmission of ARB and ARGs to humans from the environment is thought to occur through direct exposure (e.g., bathing[80–83], bioaerosols[84,85], food[86,87]), or indirectly through animal vectors (e.g., migratory birds[88], other wildlife[89 90]) and meat animal production[91–93], although there is currently limited empirical evidence showing direct transmission[94,95]. However, previous work has shown that clinically significant resistance genes originated from environmental species.

For example, Poirel et al. (2002) identified that the clinical CTX-M-type beta-lactamases originated in environmental *Kluyvera* spp. [96]. In addition to this, Poirel et al. (2005) provided evidence that the clinically significant QnrA (a plasmid-mediated resistance determinant) resides in the genome of environmental gram-negative bacteria [97]. Thus, many studies now regard the environment as a source of clinically-relevant AMR [95,98].

The current state of the empirical research investigating the direct and indirect transmission from the environment to humans is relatively limited (discussed below).

4 AMR in the Environment in the context of the WG Implementation Plan "Antimicrobial Resistance in Animals and the Environment Five Year Implementation Plan for Wales 2019-2024"

The Welsh Government has set five major components of The Five Year AMR Implementation Plan (2019-2024):

1.0 Reducing need for and unintentional exposure to antimicrobials
- *1.1 Lower the burden of animal infection – infection prevention and control. Collect/measure antibiotic usage information for each major farmed animal species in Wales. Significantly reduce the need to use antibiotics in farmed animals by raising the health status of herds and flocks, applying the principle of "prevention better than cure" through animal health planning.*
- *1.1.1 Reducing exposure of animals to infectious agents which could result in the need to treat with antibiotics*
- *1.2 Animal health planning*

2.0 Optimising use of antimicrobials in animals
- *2.1 Improving standards of antibiotic selection and prescribing*
- *2.2 Improving standards of antibiotic supply*
- *2.3 Training and education on the responsible use of medicines for those looking after livestock*
- *2.4 Providing information for companion animal owners on the responsible use of medicines*

3.0 Minimise spread of AMR through the environment
- *3.1 Improved understanding. Better understand the role of the environment in the development and spread of AMR.*
- *3.2 Responsible farm waste management practices*
- *3.3 Minimise antimicrobial contamination. To monitor antibiotics and their residues in the environment, in particular, understand how and where contamination of water occurs in order to prevent it from happening.*

4.0 Stronger laboratory capacity and surveillance of AMR in animals

5.0 Investing in innovation, supply and access to tackle AMR

The Implementation Plan is the first of its kind to address the challenge of AMR in the environment in Wales. The following section will examine the main actions and discuss the impacts on freshwaters and how they might be modified to address the wider challenge posed by ARDCs.

4.1 Source Reduction

Source reduction will reduce the prevalence of some ARGs, however, it is expected that other ARGs will persist far beyond the cessation of antimicrobial use, a phenomenon termed hysteresis[7]. Hysteresis acknowledges that the fitness cost of retaining some ARGs can be negligible, thereby allowing for their maintenance in a population well after the cessation of apparent selection[99]. Understanding the mechanisms of hysteresis, including epistasis, is a growing area of research involving the understanding of trade-offs and co-benefits at play between the genome, the organism, and the physical and biological environment over time. Owing to the relational nature of ARG selection and epistasis, it is not yet possible to accurately model and predict a specific outcome from reducing antimicrobial use – multiple outcomes are possible depending on the antimicrobial, ARG, microorganism, physical/biological environment, presence of ARDCs, and nutrient availability among other undefined explanatory variables.

The following section will discuss how source reduction of ARDCs would contribute to reducing the AMR burden in the environment.

4.1.1 Source Reduction of ARDCs in Meat Animal Production

Source reduction of antibiotics can have a positive impact on reducing the selection pressure for ARGs in the downstream environments (e.g., manure, slurry, land, and impacted freshwaters)[100]. Tang et al. (2017) reported in a systematic review and meta-analysis that animals with restricted antibiotic use had a pooled absolute risk reduction of antibiotic resistance outcomes of 10-15% (total range 0-39%) when compared to control groups. These outcomes included the presence of ARB or a change to antibiotic susceptibility in food-producing animals or humans. Reduction in antibiotic resistance outcomes depended on the antibiotic class, sample type, and bacteria under assessment[101]. Source reduction of all ARDCs (i.e., antibiotics, antifungals, antiparasitics [includes antiprotozoals and antihelminthics], metals and biocides) in farmed animals could have a more substantial impact on the co-selection risk in soil[102], air[103] and adjacent freshwater environments[104].

Metal bioavailability and toxicity is often reduced upon entry into the environment. However, unlike organic antimicrobials, which degrade over time, metals are persistent. Although metal bioavailability will vary across different environments and by metal species, metals represent a long-term AMR co-selection hazard[104,105].

The maximum authorized levels of trace elements in animal feeds have been reduced in the European Union, according to the Commission Regulation (EC) 1334/2003. Common metal supplements in animal feed and footbaths are zinc and copper [106,107]. Absorption of nutritionally provided metals will vary from animal to animal. For example, on average pigs and poultry will excrete more feed-based metals than cattle, making the manure from these monogastric animals of greater concern with respect to the impact on the environment receiving the manure[108].

A holistic assessment of total ARDCs in use within farmed animal production will offer greater insight into the hazards and drivers of AMR in the wider environment, including freshwaters. As previously described, ARDCs include many chemicals, and not just antimicrobials. By evaluating ARDCs, source reduction efforts would include metal use in feed and dips, as well as biocides and detergents. A reduction in ARDCs in animal feed would have positive implications on the future land spreading of the manure used to improve organic carbon and overall soil health.

4.1.2 Source Reduction in Non-Meat Agriculture

A comprehensive assessment of antimicrobial use in agriculture is not currently available in Wales. Fera Science Ltd. (2020), recently published a review of agricultural use of antibiotics, concluding that antibiotics, including streptomycin and oxytetracycline, have been used in the past on ornamental plants but are now banned for the control of plant diseases[109,110]. Antibacterials are infrequently used in agriculture in the UK. On the other hand, antifungals are widely used in crops throughout the UK, with azole fungicides the preferred treatment owing to their effectiveness against a broad range of fungi[111]. The evolution of triazole-antifungal resistance is primarily attributed to the widespread use of azole-based fungicides in agriculture[112,113]. Azole-fungicide persistence and selection of resistance in the environment are poorly characterised and the impact of fungicide pollution on the freshwater environment is currently without a literature base.

Vineyards are a locally relevant point for organic and metal-based antifungal use. Their use in Welsh vineyards might have implications on the antimicrobial resistance selection pressure in the vineyard [114] and adjacent freshwater bodies. The demonstration of herbicides as co-selective agents (Table 1), greatly expands on the land expected to have a transiently elevated AMR selection hazard.

4.1.3 Greywater as a Source of ARDCs in Agriculture

Greywater, defined as domestic wastewater that excludes wastewater from toilets and typically includes water from baths, showers, hand basins, dishwashers and washing machines[115], is infrequently used for irrigation in the UK, but in the likely circumstance that climate change exacerbates water scarcity, its seasonal use might become necessary. Greywater contains pathogens, ARB, ARGs and ARDCs found in the household waste stream. These are capable of AMR selection, and ARB and ARG dissemination into crops, soil and the underlying groundwater and adjacent freshwater environment[116].

4.1.4 Combined Sewer Overflows/Storm Overflows as a direct pathway for ARG and ARDC dissemination into the freshwater environment

It is challenging to quantify the extent of CSO discharge to the freshwater environment, as equipment for monitoring outfalls is frequently missing, non-operational, or the data is not publicly accessible. CSO discharges are not monitored for the volume of discharge; data will only include the time the discharge started and ended. Where data has been compiled, there is strong evidence that CSO discharge of untreated sewage is spatially ubiquitous, and temporally frequent and if not chronic. In 2020, the Rivers Trust compiled CSO discharge data[117] within England, reported in The Guardian[118], and identified that inland CSOs discharged for 1.53 million hours across the nine English water companies.

Data from 2020 showed an increase in CSO discharge incidents in England from 292,864 in 2019 to 403,171 in 2020, equating to over 3 million hours of untreated sewage entering the freshwater environment[119]. A legal duty on water companies to report CSO spills in England is expected to take effect from 2022[120]. A recent study by Hammond et al. (2021) of two WWTPs in England over the period of 2009 to 2019 detailed over 900 unreported spills of untreated sewage, 360 of which discharged into the receiving rivers for a whole day, often spilling for more than 10 full days in a row[24].

Publicly-available CSO data from Dŵr Cymru Welsh Water (DCWW) from 2018 revealed that of the 1,168 CSOs that discharged at least once in 2018, an equivalent of 39 years of untreated sewage was released into Welsh rivers. A small number of CSOs (13%, n=181 of 1,349 CSOs) did not report a spill in 2018.

Approximately three-quarters of all CSOs discharged between 1 and 99 times for a total of 6,023 days; 53% (n=709) discharging 10-99 times in 2018, for an equivalent of 5,724 days. Nine per cent of CSOs (n=123) discharged 100-199 times that year for an equivalent of 6,648 days. A small number of CSOs (n= 13), representing only 1% of all CSOs, discharged over 200 times in 2018, for an equivalent of 1,567 days.

4.2 Improve antibiotic stewardship in humans and animals

Improved antibiotic usage is an ongoing challenge in healthcare in the UK, and indeed globally[121]. Mis- and over-prescription of antibiotics is a known problem with 55% of surveyed GPs in the UK feeling pressure from patients to prescribe antibiotics and 45% prescribing antibiotics for viral infections knowing that they will have no effect[122].

A holistic view on source reduction, inclusive of all chemicals that become environmental pollutants and are known ARDCs, has not been attempted. The widespread use of biocides during the COVID-19 pandemic has been discussed as a driver of co-selection, owing to the increased use of: QACs [123], triclosan[124], chlorhexidine[125], and ethanol[126]; chlorine-based disinfectants[127]; and disinfection by-products [69,128] (Table 1).

4.3 Monitoring Spread of Antibiotics and AMR

There is no structural, statutory, surveillance dedicated to assessing the level of AMR in the environment in Wales or the rest of the UK. Monitoring the discharge of antibiotics from municipal wastewater into the environment can be used to estimate ecotoxicological and AMR selection hazards stemming from human antibiotic use[129,130]. Similar analyses can be conducted on animal manure, aquaculture and antibiotic manufacturing facilities. However, as has been previously stated, antibiotics represent a small fraction of the antimicrobials and ARDCs, all of which contribute to the overall hazard of AMR selection and maintenance in the environment. Efforts to understand ARDC pollution, not just antibiotics, will be more constructive for understanding the total ‘ARDC exposome’, i.e., the total load of ARDCs discharged to a particular location over time.

Monitoring can take two forms: 1) the culture of bacteria expressing resistance to antibiotics on growth media (phenotypic resistance), and 2) the identification of ARB and ARGs using solely molecular approaches[131,132]. Culture-based measures have previously been the gold-standard for monitoring AMR in humans, animals and the environment, but owing to their low-throughput, limited breadth (i.e., typically one species is cultured at a time and the majority of microbes are unculturable[133]) and labour-intensive nature, they are increasingly being supplemented by culture-independent techniques (i.e., qPCR and metagenomic approaches).

Molecular approaches focused on the detection of specific ARGs are typically conducted using:

- Quantitative PCR (qPCR) methods, which can be run ‘traditionally’ in a single- or multi-plex manner (i.e., one or a few genes at a time[134]) or in a high-throughput manner where over 300 ARGs can be quantified simultaneously[135]. However, qPCR methodology is limited to known resistance elements and cannot detect novel ARGs.
- Metagenomic approaches, where the entire or targeted DNA content of a sample is sequenced. Metagenomics approaches are also widely employed for understanding the breadth of antimicrobial resistance in environmental[136], animal[137,138] and wastewater settings[139]. Limitations of a metagenomic approach include: it is highly resource-intensive; it has been shown to be a less sensitive approach than qPCR [28,29] and it is much more costly. Unlike qPCR, which focuses on known ARGs, metagenomics’ strength is that it is able to identify a broad range of ARGs within a sample (depending on the sequence depth). However, ARGs are defined as genes that are genetically-similar to known ARGs so truly novel ARGs may also not be quantified. However, metagenomic data can be archived and re-analysed, enabling retrospective analysis based on future developments in computational analysis or searching for novel ARG targets.

Both qPCR and metagenomics offer insight into the presence and abundance of specific ARGs, but do not offer confirmation of their phenotype nor their host; although this latter point can be teased out of some culture-independent protocols [140,141]. The strength of a culture-based approach is the ability to characterise the genotype and phenotype of a pre-defined microorganism. It is very challenging to do this any other way. As such, where conclusive statements about a specific risk or threat are needed, culture-based methods are preferred. However, this strength is offset by the inability to focus on any other microbial threat or phenotype.

5 AMR in the Environment: Research within the UK and in Wales

As evidenced in this report, there is an extensive body of research investigating the presence of ARB and ARGs in freshwater systems globally. Similarly, there is a large global dataset reporting the concentrations of antimicrobials and other co-selective agents in various freshwater systems. There is also a growing body of evidence investigating the interaction between ARB/ARGs and antimicrobial/co-selective agents. However, this is not the case for research undertaken in Wales.

Parallels between other nations in the United Kingdom (England, Scotland, and Northern Ireland) and Wales can potentially be assumed as a result of similar population demographics, climates and infrastructure (e.g., waste management). The following sections will describe the current state of evidence on three topics from a Welsh perspective: ARB/ARGs, antibiotic residues, and transmission from the environment to humans. Where there are limited or no data relating to Wales, evidence from the UK as a whole is presented.

5.1 AMR bacteria and ARGs

Stanton et al. (Unpublished), conducted two comprehensive systematic maps during 2019-2020[94]. The first map investigated the available research evidence for the prevalence/percentage/abundance of ARB, ARGs and *intI1* (a MGE that co-correlates with elevated antimicrobial resistance[131,142]) in all natural environments, including freshwater, in all nations of the UK.

There were 29 research articles found that investigated AMR/ARGs/*intI1* in fresh and wastewater environments at various sampling sites in the UK (although none of these sites were in Wales). This ranged from investigating AMR in freshwater environments that are not heavily polluted (e.g., rivers[143,144], groundwater[145], steams, puddles[146], etc.), to highly polluted environments (e.g., untreated municipal[147,148] and hospital wastewater[149]), to environments in between (e.g., treated wastewater[143,150]). In addition, one study investigated the effect of WWTPs on rivers by measuring the prevalence of *intI1* up- and downstream of a WWTP, finding that discharge of wastewater into a river lead to an increased level of *intI1*[151]. However, Leonard et al. (2018) sampled bathing water sites in both England and Wales and tested for the presence of *E. coli* harbouring CTX-M beta-lactamases[80]. This study did not look at freshwater bathing sites, however, and instead focused on coastal bathing waters only.

As a result of similar infrastructure, population dynamics and climate, parallels can be drawn between data from freshwater environments in the other nations of the UK, but it cannot be assumed that freshwater environments in Wales will show similar patterns of AMR, ARGs, *intI1* and pollution as the rest of the UK. The relative role of closed mines on freshwater in Wales remains uncharacterised with regard to its impact on AMR. Given the reported 1,300 abandoned metal mines in Wales, impacting an estimated 200km of river reaches, the impact of metal mine water pollution on AMR remains a potentially important knowledge gap[152].

5.2 Antibiotic residues

The German Environment Agency (Umweltbundesamt) has published and regularly updated a database titled “Pharmaceuticals in the environment” which collates measured environmental concentrations of antibiotics and other pharmaceutical drugs from other published research from all over the world[153]. The latest database, published in 2019, can be filtered to show antibiotics detected in freshwater environments in Wales. Using this filtering tool, a total of 217 records are reported. Although this number seems significantly higher than the reported number of studies for ARB/ARGs found in Wales in the unpublished systematic map by Stanton et al., these records correspond to multiple different antibiotic compounds reported in only three separate publications[154–156]. These concentrations range from 87 records where antibiotic concentrations were below the limit of detection, to the highest record of 12,397 ng/L for sulfapyridine in untreated wastewater (Coslech

WWTP, River Ely)[154]. The resulting average concentration of all antibiotics found in all freshwaters in Wales reported in this database is 443 ng/L. Whilst this number is relatively low, one study has shown selection for resistance can occur at antibiotic concentrations as low as 400 ng/L [28], which suggests that this average and the range of antibiotic concentrations found in the database could be selecting for resistance. However, this will depend on the type of antibiotic, as different antibiotic compounds have been shown to select for resistance at different concentrations [27,28]. These values do not include those in environments that are indirectly impacting freshwater, such as manure, which may leach into waterways.

5.3 Transmission

Based on the results from the unpublished systematic map[94,157], transmission is presently an extremely under-researched area. Globally, there is limited empirical evidence of transmission from the natural environment to humans. Research tends to focus on estimated exposure risk[158–160], cohort studies[161], case control studies[162] or descriptive studies of isolated ‘accidents’ resulting in a resistant infection[163]. The unpublished systematic map found 39 studies, globally, investigating empirical evidence or exposure risk estimates for transmission from any environmental settings to humans and 14 of these were from freshwater or wastewater environments.

Considering there are only limited studies globally investigating transmission, four studies have investigated this in Wales (which equates to ~10% of global research). Although all of these studies investigate water environments, they do not investigate freshwater or wastewater, but focus on tap water[162,164] and coastal water[80,165]. In addition, there are no publications to be found investigating transmission of AMR from fresh or wastewater to humans in any of the other three UK nations.

6 Solutions to Direct & Indirect Drivers of AMR and their Co-Benefits and Trade-Offs in Freshwaters

Here we present an outline of the direct and indirect drivers of AMR in (fresh)water environments and possible interventions, which are non-exhaustively assessed for co-benefits and trade-offs (Table 2).

Table 2 Direct and indirect drivers of AMR and possible interventions

Intervention	Type	Time frame	Co-benefits	Financial cost	Key trade-offs
Direct Drivers					
Improved WWTP treatments (e.g. increased treatment time)	Direct	Short	Reduces overall load of other pollutants (e.g. ammonia, phosphorus, BOD, organic pollutants)	High	Increased cost per volume of sewage.
Improved WWTP treatments (e.g. UV, Ozonation)	Direct	Short	Reduce risk of ecotoxicological hazard from ARDCs on aquatic life	High	UV is differentially effective for different ARB/ARGs. UV reduction significantly reduces bacterial load but can increase the proportion of ARB due to co-selection processes linked to the selection of UV resistance. Increase in carbon footprint of WWTP.
Improved WWTP treatments (e.g. coagulation)	Direct	Short	Improved quality of discharged water	Medium	Increase in metal loading in sludge will have implications for the utility of sludge in landspreading
Indirect Potable Reuse	Direct	On-going	Removal of the chemical and biological hazards from wastewater. Generation of valuable product: drinking water	Very high	Significant cost. Societal acceptance. Impact on rivers reliant on sewage for flow. High cost is offset by the production of a valuable end product.
Improved sewage network infrastructure (e.g. storm overflows, combined sewer overflows)	Direct	Long	Improvement of water quality and habitat. Reduced exposure for humans, companion animals (e.g. dogs) and wild animals	High	Significant financial, societal and carbon costs
Reporting on combined sewer overflows	Direct	Short	Allows for tracking of progress and better estimation of environmental impact and dissemination and transmission risks	Low	Cost, impact on perception of water sector
Sewage sludge / Biosolids / Anaerobic digestion / Pyrolysis	Direct	Short	Treatment of sludge: reduction of the load of all pollutants (except metals). Advance oxidation, anaerobic digestion & pyrolysis can generate energy.	Medium	Metals will be concentrated in digestate which could impact rate of ARG loss.

Intervention	Type	Time frame	Co-benefits	Financial cost	Key trade-offs
Direct Drivers					
Preventing direct access of farmed animals to water courses by fencing	Direct	Short	Reduces pollution load in river and eutrophication risks.	Low	Cost, demands alternative water source for animals
Both Direct & Indirect Drivers					
Reduction of use of antimicrobials in agriculture and aquaculture	Direct & Indirect	On-going	Requirement of higher biosecurity and/or lower herd density to manage pathogen risk could lead to improved animal welfare	Low-Medium	Risk of higher cost of animal production
Indirect Drivers					
Reduction of clinical consumption	Indirect	On-going	Fewer misdiagnoses, misprescribing. Potential for introduction of better preventative medicine.	Medium-High	A carefully considered engagement plan must be implemented to ensure the transition does not harm patients. Improved and robust evidence base supporting e.g. shorter durations of antibiotics for common conditions such as urinary tract and respiratory infections
Addressing groundwater Ingress & Implementing Sustainable Urban Drainage	Indirect	On-going	Reduction of water entering combined sewers will increase capacity for heavy rainfall events. Reduction of road runoff will improve freshwater quality and could positively impact aquatic life.	High	Initial investment cost of sustainable urban drainage is high. High cost is offset by some SuDs solutions, e.g., ponds, which offer high natural capital.

Future work should also include the economic costs of implementation at scale. It is only after the costs of implementation are estimated that the costs averted, co-benefits and trade-offs of each of the interventions can be properly contextualised and an informed decision can be made. It is likely that the co-benefits and trade-offs will change within a context of a changing climate and growing population.

6.1 Solutions to Major Direct Drivers

6.1.1 Wastewater Treatment Plants

WWTPs are critical infrastructure that receive antimicrobial-resistant microorganisms directly from the human gut, along with a portion of all the pharmaceuticals that were consumed and those incorrectly disposed of through the toilet. Industrial effluent of a varied nature, possibly including antimicrobial waste, comes together with the human-derived ARB and ARGs in the bioreactors we call WWTPs. Each WWTP represents a unique and dynamic system for the selection and dissemination of ARB, ARGs and ARDCs into the environment through treated and untreated effluent and sludge[166]. There are numerous measures that can be put into place to reduce the load of ARB, ARGs and ARDCs discharged into the environment, as discussed below.

6.1.2 Increasing Treatment Time

Increasing the time sewage is treated (i.e., hydraulic retention time) will positively impact the removal of ARDCs, thereby reducing their load being discharged into the environment[167]. A reduction in the ARDCs will reduce the selection pressure that wastewater represents after discharge to the environment. WWTPs will tend to reduce ARGs by 2-3 log from untreated sewage levels[167], a reduction that is likely to increase further with increased treatment time.

Timeliness: In some cases, increasing the hydraulic retention time is infeasible without significant upgrades to the WWTP; this would need to be considered on a case-by-case basis. A > 5-year horizon for implementation might be feasible, where upgrades are needed. Where upgrades are not needed, it can immediately be implemented.

Co-Benefits: It is well established that longer residence times will reduce the overall load of pollutants discharged from wastewater (e.g., ammonia, phosphorus, BOD, organic pollutants) with positive implications for micro-, meso- and macro-aquatic life downstream of the WWTP discharge point.

Trade-offs: Longer treatment times will come with increased operational costs to treat the same volume of sewage. In the absence of upgrades to the WWTP, the increased treatment time will impact the amount of wastewater that can be treated per day. Increasing hydraulic retention time could face significant costs in upgrading many WWTPs to accommodate the added volume. The possible impacts of higher hydraulic residence times on AMR and ARG selection within the treatment works is not well understood, and further research is needed to understand the possible trade-offs against the benefits of ARDC reduction.

6.1.3 Advanced Oxidation & Sterilization

Advanced oxidation, UV, chlorine, and ozone treatment of treated wastewater are effective in reducing the load of ARGs and ARB, and in some cases ARDCs[168–170]. The expense associated with these interventions can be more effectively justified through consideration of its co-benefits. The use of advanced oxidation/sterilization approaches has the added benefit of reducing the hazard associated with the effluent making it more suitable for catchments where there are recreational uses of the river (e.g., bathing), or when the river feeds into a shellfish bed or coast with bathing water status.

Timeliness: The timeline is relatively short for implementing many of these technologies, estimated to be a mitigation that could be implemented within 5 years.

Co-Benefits: The removal of ARDCs from the wastestream would alleviate the ecotoxicological hazard they pose in the receiving river with possible impacts on aquatic life. The increased interest by the UK public in using rivers as recreational bathing waters will necessitate dramatic reductions in pathogen loads that could be provided by advanced oxidation/sterilization[171].

Trade-offs: The range of solutions represented by advanced oxidation/sterilization all come at a significant running cost with implications to the carbon footprint of the WWTP. UV treatment has been shown to be differentially effective across microorganisms and different ARGs, making it a beneficial complementary approach that is unlikely to be useful as a sole mitigating measure[172]. There is evidence that although UV disinfection significantly reduces total bacterial survival after treatment, the proportion of ARB can increase due to higher tolerances of resistant bacteria to UV compared to non-resistant bacteria[170]. Additional evidence for chlorine-stimulated HGT within surviving microorganisms might lead to unforeseen, undesirable outcomes that favour, rather than reduce, the selection of ARB and ARGs[59].

6.1.4 Coagulation

The reduction of ARB through the flocculation of treated sewage prior to discharge offers an alternative to sterilization. The precipitation of flocs (larger clusters) of bacteria in settlement tanks prior to their discharge will reduce the abundance of ARB discharged from WWTPs. Although feasible, it is not standard practice to recycle the chemical coagulant (iron or aluminium). If recycling was achieved, the life cycle for such an approach would become more sustainable. There are few studies on the use of coagulation for the removal of ARGs, but limited evidence suggests modest removal levels of 0.5-3.1 log reduction[173].

Timeliness: Coagulation could be implemented in a relatively short timescale (<5 yrs).

Co-Benefits: Coagulation will improve the quality of the discharged wastewater and likely have a positive impact on shellfish beds and bathing waters that rely on low pathogen discharges from upstream WWTPs.

Trade-offs: The added cost of coagulation and the potential increase in metal loading of sludge will have implications on the utility of the sludge for landspreading.

6.1.5 Indirect Potable Reuse (IPR)

The transformation of wastewater into high quality recycled potable water is increasingly common worldwide, but is primarily implemented to address severely water-stressed populations[174,175]. Given all highly effective solutions to reducing AMR in the environment are going to be expensive, it is important to evaluate the additional benefits of approaches such as IPR, which not only removes the ARB problem but also produces a valuable end product, namely drinking water. The long-term value to society and the environment of implementing a holistic solution such as IPR will become increasingly obvious as climate change progresses to longer, dryer periods and the population continues to grow.

Timeliness: IPR is being adopted in increasing numbers of locations globally. It has a long delivery horizon, but represents a holistic solution that is maximally future-proofed.

Co-Benefits: The co-benefits to the environment from zero discharge are difficult to quantify. The complete removal of all AMR, pathogen and chemical risks originating from WWTPs will have the greatest positive impact on eliminating the spread of AMR in the aquatic environment, with likely significant co-benefits to aquatic life, bathers and shellfish beds.

Trade-offs: It is unclear what the impact would be on receiving rivers if sewage did not discharge to them, as the flow in some rivers and streams can contain a significant proportion of sewage effluent discharge at certain times of the year. The cost of achieving such an endpoint needs to be assessed and compared against the cost of increasing treatment retention time, sterilization and coagulation approaches. The cost implications of implementing IPR could be completely offset by the co-benefits to human and environmental health and the provision of a sustainable source of drinking water. The benefits of IPR will become increasingly more tolerable in the context of a changing climate where rainfall is more irregular and summers are dryer. A partial IPR system, i.e., some treated sewage is still released into the river, might alleviate some of these trade-offs.

6.1.6 Combined Sewer Overflows (CSOs)/Storm Overflows

The challenge of CSOs in Wales and the wider UK stems from numerous concurrent factors: 1) underinvestment in the sewerage network[176,177]; 2) network capacity not expanding with the population[178–181]; 3) continued use of combined sewers[181,182]; 4) insufficient implementation of sustainable urban drainage schemes[183]; 5) climate change generating heavier storms[181,184]; 6) leaking pipes allowing groundwater ingress[177,179]; and 7) under-capacity storm tanks at WWTPs[177,185]. These factors conspire to fill sewer networks with water even during light rain. All of these factors need to be addressed to eliminate the need for CSOs[181,186]. Prioritising these factors in an AMR mitigation plan would be justified given the enormous role that CSOs play in the dissemination of pathogens, ARGs and ARDCs. Regular reporting on the frequency and volume of CSO discharge will facilitate tracking progress over time and to improve on our understanding on the risks to the receiving environment as well as dissemination and transmission risks.

Timeliness: Addressing the many factors contributing to CSOs will be a >10-year effort, but is already being recognised as a high priority by the regulators[180] and the water industry[187].

Co-Benefits: The elimination of CSOs has the potential to improve the quality of impacted river water quality and habitat. The risks to human health may also be alleviated, particularly wild swimmers who can be exposed to sewage-impacted rivers[188]. Dogs and other animals that live in/by or use rivers may also have a reduction in pathogen exposure.

Trade-offs: There is a significant financial, societal and carbon cost associated with addressing CSOs and their upstream drivers in the form of: 1) installation and retrofitting sustainable urban drainage throughout cities, town, motorways and roads; 2) reducing water usage through voluntary reductions by society; 3) infrastructural upgrades that might temporarily impact services across the UK; 4) planning permission for homes would not be granted unless SUDs and existing infrastructure were fit for purpose, and 5) the increase in operational and carbon costs associated with the treatment of wastewater previously discharged by a CSO.

6.1.7 Fate of Biosolids/Manure/Anaerobic digestion/Pyrolysis

We have found 55.5% of headwater streams in the national GMEP survey were not fenced, meaning free access to the waterbody by livestock[189] . A lever for encouraging landowners with livestock to fence-off access to waterbodies would alleviate some of the risks from AMR that originate from livestock faeces.

In the more managed/engineered environment of a wastewater treatment plant, sewage sludge and biosolids are produced as by-products of treatment. In lieu of landspreading of the biosolids, anaerobic digestion of sludge can be a viable way to extract energy from the wastestream; a similar approach can be applied to animal waste (e.g., manure and slurry). It remains relatively poorly understood how effective anaerobic digestion is in removing ARGs and ARDCs. Current evidence supports the use of thermophilic digestion as it operates at temperatures much higher than the human gut, which consequently reduces the prevalence of gut commensals and pathogens and the downstream hazards posed by land spreading of biosolids, manure and digestate[190]. Welsh Water has moved to the anaerobic digestion of >95% of the sludge produced in Wales in Advanced Anaerobic Digestion hubs[191]. The residual risk posed by the digestate to the spread of ARGs and ARDCs remains poorly uncharacterised. Residual metals in digestate can help to sustain high ARGs and MGEs[192], lending support to source reduction of metals, particularly in animal feed, which would alleviate this hazard in digestate.

Pre-treatment stages of anaerobic digestion, including acid phase digestion, sonication, thermal hydrolysis and enzymic hydrolysis, are being trialled and are operational in the UK[190]. Thermal hydrolysis is the most popular method, which applies pressure (120-130 psi) and temperature (165°C) for about 30 minutes to hydrolyse the solids prior to digestion thereby reducing the ARG load and some ARDCs prior to anaerobic digestion. To date, there are no studies on the effects of pre-treatment of anaerobic digestion feedstock and subsequent digestion on ARGs and ARDCs. Such studies would aid in assessing the hazard such digestate poses to the terrestrial and adjacent aquatic environment.

Pyrolysis of sludge/biosolids/digestate represents a relatively new solution to the challenge posed by these waste products to the spread of ARGs and ARDCs[193]. Pyrolysis is most frequently achieved at 400C, thereby eliminating all ARGs and most ARDCs. Metals will remain within the biochar that remains, but is largely immobilised within the matrix and likely poses a reduced risk to leaching and ARG selection[193]. A review of available information on the application of pyrolysis/gasification in the UK in 2019 indicated that no full-scale pyrolysis/gasification plants using sewage sludge as a sole or majority feed source existed in the UK[193].

Timeliness: Pre-treatment, anaerobic digestion and pyrolysis are currently accessible options for mitigating the hazard posed by ARGs and ARDCs in sludge and manure.

Co-Benefits: Treatment of biosolids and manure prior to land application can potentially reduce the environmental load of all pollutants, except metals. Advanced oxidation, anaerobic digestion and pyrolysis can generate energy from sewage and farm waste that offers ways of offsetting the additional cost of treatment.

Trade-offs: There will be a cost to dewatering the sludge to make it cheaper to transport, as well as the infrastructural costs associated with anaerobic digestion – including land. The net carbon budget over the life cycle would need to be calculated to determine if this represents a co-benefit or trade-off. Metals will be concentrated in the digestate which could impact the rate of ARG loss and alter the resistance profile to one predominantly driven by metals.

6.1.8 Meat Animal, Dairy and Egg Production

Annual reporting on ARDCs will identify environments/settings where particular chemicals might currently be over- or mis-used. Implementation of modest targets for ARDCs will enable quick wins, as was seen in the implementation of antibiotic targets in animals following O'Neill's AMR Review (i.e., a reduction in antibiotic use in livestock and fish farmed for food to a multispecies average of 50 mg/kg by 2018 (from the most recent 2014 figure of 62mg/kg) using methodology harmonised across other countries in Europe)[194,195]. There have been efforts to reduce some metal supplements in animal feed in European law, however, it is unclear how this translates to the UK setting. A concerted reduction in metal use will be a key source reduction measure that can reduce co-selection within the animal, manure, and anaerobic digester which will translate to lower hazards from ARGs and the accumulation of metals in the environment.

Timeliness: Source reduction of antibiotics in meat animal production is ongoing, however, efforts to reduce it as close to zero without compromising animal welfare is the goal. Efforts to source reduce ARDCs in this sector are immediately capable of being implemented, however, the ability to compensate for the benefits enjoyed by some ARDCs might have longer timelines, i.e., finding alternatives to metal supplements in animal food.

Co-Benefits: Source reduction of antimicrobials and ARDCs will likely require higher biosecurity and/or lower herd density to manage the pathogen risk; such a shift could translate into improved animal welfare.

Trade-offs: There is a risk that a reduction in the use of chemicals for meat animal production would come at a higher cost due to the need for reduced flock/herd size and/or substantial improvements in biosecurity, both of which can impact on the cost of animal production.

6.2 Solutions to Major Indirect Drivers

6.2.1 Source reduction of antimicrobials in humans and animals

A reduction in antimicrobial prescriptions can be achieved through improved infection prevention and control (i.e., use of personal protective equipment (PPE)), more effective roll-out of existing and new vaccines, and more effective roll-out of existing and new diagnostics for evaluating the aetiology of disease and the susceptibility of the infecting pathogen to antimicrobials. A reduction in human and animal antimicrobial use could greatly reduce the load in the waste stream with concomitant reductions in the hazard they pose to AMR selection in the WWTP, the receiving rivers and on land following landspreading[129].

Timeliness: Reductions in antibiotic prescribing are ongoing within the NHS and significantly greater reductions are achievable in the near term, given adequate investment. The COVID-19 pandemic has highlighted the importance of infection prevention and control measures in the healthcare and residential/care home setting, which if maintained, could potentially contribute to a significant reduction in healthcare-associated infections and subsequent antimicrobial use.

Co-benefits: All the solutions to reducing antimicrobial use have implications for fewer infections and better well-being/animal welfare; fewer misdiagnoses and fewer incidents of antimicrobial mis-prescribing and side effects. Leveraging the success of the COVID-19 pandemic vaccine will likely make future measures of preventative medicine easier to sell to the public.

Trade-offs: Efforts to change the status quo will necessitate a carefully considered communication and engagement plan with all stakeholders as a poorly considered transition could mean that few people benefit from the advances.

6.2.2 Groundwater Ingress & Sustainable Drainage Systems (SUDs)

Rainwater and road runoff represent an increasing quantity of water entering the already straining sewage networks. All sustainable urban drainage efforts to reduce the amount of water entering the sewer network will alleviate this pressure leading to fewer CSO and storming events[196]. SUDs will address the challenge stemming from rainfall events, however, they will not address the challenge posed by groundwater ingress. Groundwater poses a chronic problem in areas where leaking pipes cohabit areas of high groundwater, a phenomenon that might be more common during the winter wet period, but not exclusively. The combination of high groundwater ingress and poor SUDs relies on the CSOs to avoid sewage entering the streets and people's properties; under these circumstances sewage must bypass WWTP treatment as flow to full treatment cannot be exceeded[24]. Less groundwater and rain in the sewer network will make it easier for existing infrastructure to cope with the current load of sewage thereby improving the quality of water downstream of CSOs and WWTPs, including pathogens, ARGs and the selection pressure from ARDCs[183].

Timeliness: Reducing groundwater ingress remains an ongoing activity of the water industry, however, solutions are not keeping pace with the need. The capacity to implement SUDs in the UK is high and can be immediately implemented at a much larger scale with governmental prioritisation.

Co-benefits: A reduction in the quantity of water entering combined sewers will increase the capacity of existing infrastructure to tolerate heavier rainfall events, which are going to be more common in a changing climate. Reduced road runoff entering freshwater environments will improve water quality and could have a substantial impact on highly sensitive aquatic life. For example, a recent study in the Northwest of the United States found a chemical component of tyres was found in the runoff from roads, which played a critical role in the survival of migratory fish species in impacted rivers[197].

Trade-offs: SUDs require an initial investment and many of the solutions that act as interceptors of road-runoff will need periodic maintenance. The land that many SUDs solutions require might translate to higher costs for house building.

7 Knowledge Gap Analysis

7.1 Welsh data

As evidenced in Section 5 (p.15), there are a significantly limited number of studies investigating ARB, ARGs and antibiotic concentrations in Welsh freshwater systems. In fact, there is only one study identified investigating ARB/ARGs (researching coastal bathing waters[80]). In addition, according to the Umweltbundesamt "Pharmaceuticals in the environment" database published in 2019, only two studies investigated antibiotic concentrations in Welsh freshwater[153].

As suggested above, parallels can be drawn between data on freshwater from other nations in the UK and Wales, as they have a similar climate, infrastructure and population dynamics. However, a lack of basic surveillance data in Welsh freshwater environments means that there is no empirical evidence to show the extent of the problem. It is also feasible that there are unexplored aspects of environmental AMR that are more important in a Welsh context, for example, metal pollution from abandoned mines, and a wetter climate which could impact farm and road runoff, and CSOs discharge frequency [152]. Also with time, policy priorities between the four nations of the UK may change, and mechanisms such as the Sustainable Farm Scheme or a change in the regulatory floor may result in further divergence, This makes it difficult to propose a plan of action to tackle the problem. Without data specific to Wales, it is difficult to know whether the interventions recommended in Section 8 (p.25) would be enough to reduce the load of ARB/ARGs/antibiotic concentrations, or may in fact be unnecessary as levels could be lower than those in comparable environments in England, Scotland and N. Ireland. Without addressing this knowledge gap, informed, evidence-based decisions about the best plan of action to tackle AMR in freshwater environments, specifically in Wales, are difficult to make. In addition, without this knowledge it is unknown whether Welsh freshwater environments that humans interact with pose a risk to human health through transmission from the environment. An integrated approach to understanding the linkages between source, pathway and receptor will be fundamental to ensuring that policies and approaches are not made in silos.

7.2 Global data

In contrast to research in Welsh freshwater, there is a wealth of data investigating ARB/ARGs/antibiotic concentrations in the environment on a global scale, particularly in heavily polluted environmental niches. However, there are significantly fewer data showing empirical evidence of transmission from the natural environment on a global scale. The lack of this type of evidence means that it is unclear the risk of acquiring ARB and ARGs by interacting with various types of natural environments, including freshwater.

As noted in Section 5 (p.15), a systematic map[157] found only 14 studies investigating fresh and wastewater from global literature. In addition to cohort studies, these publications included a number of risk assessment studies and descriptive studies of isolated accidents resulting in a resistant infection. Proving that a particular species of bacteria with a particular ARG is regularly transmitted from a specific natural environment, such as fresh and/or wastewater, to humans and that this transmission results in either colonisation or infection, is difficult. As a result, literature on this topic to date is sparse.

8 Recommendations

The evidence reviewed for this report supports the following recommendations for reducing the environmental burden and hazard posed by antimicrobial, ARG and ARDC pollution in Wales.

A. ***Source reduction of ARDCs***

Antibiotics represent a large mass of drugs used in the UK with an established role in driving AMR, but antibiotics are a relatively small component of all ARDCs in use, notably other antimicrobials (particularly antifungals), metals and detergents/biocides.

The use of antimicrobials, metals and biocides in home, industry and meat animal, dairy and egg production has not been challenged with a source reduction effort. A risk ranking study should proceed prior to any concerted source reduction effort to ensure the most impactful components and sources are targeted. Notably, source reduction can be made through indirect actions such as vaccinations and personal protective equipment (PPE) with both reducing the risk of infection and subsequent antibiotic use.

B. ***Eliminate the need for combined sewer overflows***

Elimination of CSOs is aspirational, but should be prioritised as they represent one of the largest chronic sources of ARB, ARGs and ARDCs in the freshwater environment. Because the drivers of CSOs are so complex, it is one of the most challenging AMR mitigating actions to solve.

Reducing CSO discharges will require coordinated action that involves: builders, planning permission, water industry, sustainable urban drainage, and a sensitivity to climate change and population growth. The problem will also necessitate innovation in how to address leaking, ageing and under-capacity sewer infrastructure, and a plan for WWTPs to accommodate storm water capacity commensurate with future heavy rainfall estimates. Reporting on the frequency and volume of CSO discharge will be instrumental in tracking progress and developing approaches for assessing the risk of AMR dissemination and transmission.

C. ***Reduce the ARG/ARDC load in sewage and farm waste***

Human and farm waste represent a continuous, chronic stream of antimicrobials, ARGs and ARDCs. Source reduction is central to mitigating AMR in the environment.

Technological solutions are available to treat contaminated waste streams, such as pyrolysis, advanced oxidation, thermophilic anaerobic digestion, but they come at a cost, which might be substantially lower after source reduction was implemented. There is a need to better understand the implications of land spreading of sewage and farm waste for selection, dissemination and transmission from the environment to humans and animals. Such an understanding will increase greater circularity and other co-benefits, e.g., improved soil health and reduced greenhouse gas.

D. ***Improve wastewater treatment and the removal of ARB, ARGs, and ARDCs***

Because CSOs are ubiquitous in the UK, it is impossible to know the extent to which treated sewage is responsible for ARG selection and dissemination in freshwaters. Advanced treatment options to reduce or remove ARB, ARGs and ARDCs will very likely be necessary given the challenge that source reduction poses.

More research is needed to better understand the effectiveness of different wastewater treatment technologies in both reducing the development and dissemination of ARB and ARGs and how widespread these effective technologies are within Wales, already.

E. ***Establishing Discharge Endpoints for ARDCs and ARGs***

In the absence of empirically-derived no-effect concentrations for every antibiotic against all microorganisms, a precautionary approach aligning with the AMR Industry Alliance guidelines for antibiotics can be established for all antimicrobials at a concentration of 0.1 µg/L in effluent[26,198].

These endpoints would be revised following empirical research to indicate the threshold should be higher or lower. A precautionary approach is desirable as it gives clear targets for all antimicrobials, which is more tractable from the perspective of a wastewater engineer than having hundreds of different endpoints. The reality of the engineering challenge is that the lowest endpoint will be the driver of the technology employed.

F. ***Establish routine freshwater and wastewater monitoring***

Monitoring of the direct drivers of AMR to freshwaters will provide the baseline needed for determining if the aforementioned AMR mitigating measures have been effective. It remains an open question as to the most informative way to monitor AMR, however, a mixture of molecular and culture-based methods currently offer a desirable balance. Culture-based methods offer an understanding of very specific risks, such as extended-spectrum beta-lactamase (ESBL) producing *E. coli* in aquatic ecosystems, whereas molecular approaches provide a breadth of understanding that is more suited to high-throughput. Careful consideration needs to be given to the representative nature of any monitoring effort given the highly variable nature of wastewater and freshwater systems. An integrated approach to understanding the linkages between source, pathway and receptor will be fundamental to ensuring that policies and approaches are not made in silos.

G. ***Research Knowledge Gaps***

- How will climate change (i.e., dryer summers and wetter winters, with heavier periods of rain), changes and increases in meat animal and animal product production, and human population growth impact the environmental dimension of AMR, in Wales?
- Develop quantitative metrics for the hazard posed by mixtures of ARDCs.
- Improve our understanding of the ARG/ARB transmission risks from different environmental compartments to humans and animals.

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