Antimicrobial Resistance In Relation To The Environment In Scotland

Literature Review

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DISCLAIMER

The views expressed in this report are those of the researchers and do not necessarily represent those of the Environmental Standards Scotland. The authors have no conflicts of interest to declare.

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ABBREVIATIONS

AMR	Antimicrobial Resistance
AMS	Antimicrobial Stewardship
AMU	Antimicrobial Use
ARB	Antimicrobial Resistant Bacteria
ARHAI	Antimicrobial Resistance and Healthcare Associated Infection (Scotland)
ARG	Antibiotic Resistance Gene
CREW	Centres of Expertise for Waters (Scotland)
CSO	Combined Sewer Overflow
ESPAUR	English surveillance programme for antimicrobial utilisation and resistance
FAO	Food and Agriculture Organization of the United Nations
FSS	Food Standards Scotland
MGE	Mobile Genetic Elements
OHBP	One Health Breakthrough Partnership (Scotland)
PATH-SAFE	Pathogen Surveillance in Agriculture, Food and the Environment
PHS	Public Health Scotland
qPCR	Quantitative Polymerase Chain Reaction
SAPG	Scottish Antimicrobial Prescribing Group
SEPA	Scottish Environment Protection Agency
SEFARI	Scottish Environment, Food and Agriculture Research Institutions
SOHAR	Scottish One Health Antimicrobial Resistance Register
SOHNAAP	Scotland One Health National Antimicrobial Resistance Action Plan
SONAAR	Scottish One Health Antimicrobial Use and Antimicrobial Resistance
UNEP	United Nations Environment Programme
VMD	Veterinary Medicines Directorate
WGS	Whole Genome Sequencing
WHO	World Health Organization
WOAH	World Organisation for Animal Health
WWTP	Wastewater Treatment Plant

1. INTRODUCTION

This report presents the findings of a literature review on antimicrobial resistance (AMR) in relation to the environment in Scotland. It was commissioned by Environmental Standards Scotland (ESS) following the publication of the Baseline Evidence Review (Environmental Standards Scotland, 2022) which identified a need to develop a better understanding of the causes, impacts and controls of AMR in Scotland to support the future analytical priorities of ESS.

This review is structured as follows. Section 1 presents a brief background to the growing problem of AMR in the environment and outlines the aims of the review. Section 2 describes the methodology used to achieve these aims. Section 3 presents the findings of this review, specifically of the causes, impact and controls of AMR in relation to the environment in Scotland. Conclusions that can be drawn in relation to the set review questions are provided in Section 4. The review also considers gaps in the available literature and provides an overview of the ongoing projects, which may help fill evidence gaps or assist consideration of whether further research is needed.

1.1 Background

In recent years, AMR (the ability of microbes to resist the effects of drugs including antibiotics, antivirals and antiparasitics) has been recognised as a significant threat to human health and the global economy, with the highest burdens in low-resource settings (World Health Organization, 2001). An estimated 4.95 million deaths worldwide were associated with bacterial AMR in 2019 (Murray *et al.*, 2022). This figure is predicted to rise to 10 million deaths annually by 2050, with a significant corresponding impact on the global economy of approximately 100 trillion USD if no action is taken (O’Neill, 2014). AMR also threatens the health of animals and plants grown for food, with effects on food security, food safety and the environment (FAO *et al.*, 2022). Consequently, many national and international bodies are focused on understanding how best to control AMR proliferation and mitigate its effects.

The environment has been identified as the single largest source of antimicrobial-resistant bacteria (ARB) and genes (ARG), with environmental microbes described as the “wellsprings of resistance elements” (Surette and Wright, 2017). This resistance has been developed over billions of years of evolution, to enable microbes to survive in the presence of substances that are toxic to them, such as inorganic metals and organic compounds. The environment can also provide a number of pathways through which AMR, that has arisen from human activity, can spread (Larsson *et al.*, 2023).

1.2 Aim of the review

The overall aim of this review was to produce a clear and concise overview of the available literature on AMR to help develop a better understanding of the resistance issue, its controls and impacts, in relation to the environment and how that applies to Scotland. The specific review questions were:

1) **Cause:** There is a general understanding of the common causes and pathways of AMR genes and AMR-carrying bacteria into the environment. How does this apply in a Scottish context?
    a) What are the known causes of AMR in the environment & what is understood about their relative importance?
    b) What are the projected causes of AMR?
    c) What are the nature of these?
    d) What is known about the scope, scale and frequency of these?

2) **Impact:** What are the known impacts of AMR in the environment, and how does this apply in a Scottish context?
    a) What is the nature, scope, scale and frequency?
    b) Can we quantify the level of current and future risk from these impacts?
    c) How does this compare across the UK and Internationally?

3) **Controls:** What control mechanisms are in place around the use and introduction of AM (antimicrobials) / AMR?
    a) What is the type/ use/ efficacy of these controls?

b) Are any of these specific/ tailored to Scotland?
c) How does this compare nationally/ internationally?
d) If control mechanisms are not in place, are they expected/ forthcoming?
e) Is it known what the potential impact of changes to existing control mechanisms could be?

2. METHODS

A rapid review of the literature was undertaken over a period of 35 days during September and October 2023. Rapid review methodology utilises similar processes to a full systematic review but generates a more timely synthesis of the evidence. It does this by limiting scope (e.g., search terms and inclusion criteria) and various aspects of synthesis (e.g., data extraction and bias assessment) (Ganann, Ciliska and Thomas, 2010).

2.1 Scope of the review

In order to ensure a manageable but targeted scope that could address the review questions, we: (a) adopted a One Health lens, which recognises the interdependence of human and animal health with the environment (WHO, 2021a) and (b) developed a set of inclusive definitions that could structure the search, selection and synthesis of the identified literature. The key definitions are outlined below.

One Health – a cross-disciplinary approach that recognises the interconnection between the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) (WHO, 2021a). Those taking a One Health approach, design and implement programmes, policies, legislation and research across multiple sectors to tackle public health problems, including AMR.

Ecosystems – are defined as “natural communities of organisms, the physical environment with which they interact and their functioning as an ecological unit – mostly unaltered by anthropological land uses” (WHO, 2022).

Environment – the term environment includes "the complex mixture of physical, chemical and biotic factors, including land, air, water, soil and all living things that interact within it, as well as areas that are natural ecosystems or transformed to various degrees by humans (urban, agricultural and so on) (WHO, 2022)".

Environmental compartments – reservoirs and transmission pathways of AMR in the environment, including water (surface water, groundwater and drinking water), soil and sediment, air (dust, bioaerosols), and wildlife[1] (Singer, Fry and Antoniou, 2020).

Health of Environment – defined as "the extent to which the environment is able to function, maintain biological and chemical processes, adapt to change or cope with the impacts of human activity" (FAO *et al.*, 2022). It determines its state and its ability to function at its best, including how free it is from non-native pollutants (WHO, 2022).

Antimicrobial Resistance (AMR) - a natural occurrence when microorganisms such as bacteria, viruses, parasites or fungi become resistant to antimicrobials treatments to which they were previously susceptible. AMR refers to AMR genes (ARG) and AMR-carrying bacteria (ARB).

Antimicrobial (AM) - an agent intended to kill or inhibit the growth of microorganisms. They include antibiotics, fungicides, antiviral agents and parasiticides. In addition, some heavy metals, disinfectants, antiseptics, and many other pharmaceuticals and some natural products may also have antimicrobial properties (UNEP, 2023).

Antibiotic Resistome – the name given to the collection of antibiotic resistance genes (ARG) in the environment, their precursors, and some potential resistance

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[1] **Wildlife** – non-domesticated animals, including invertebrates, wild fish and seafood animals, living in a natural environment, sometimes hunted for food (game species)

mechanisms within microbial communities (Surette and Wright, 2017). The genetic elements that cause resistance within a microbe can either be intrinsic (inherited) or extrinsic (acquired). These acquired genes may be integrated into the genome of the microbe or can exist as mobile genetic elements (MGEs), such as plasmids, transposons and conjugative elements. Horizontal gene transfer (HGT) of resistance genes can take place via three mechanisms:

- Conjugation - transfer of DNA between bacteria by direct cell-to-cell contact, considered the main AMR dissemination strategy.
- Natural transformation - uptake of extracellular or free DNA from the environment.
- Transduction - transfer of DNA between bacteria via a bacteriophage (a virus that infects and replicate within bacterial cells).

Antimicrobial pollution - Pollution containing antimicrobial agents (e.g. waste streams from households, hospitals, agricultural, and chemical manufacturing), which disrupts the microbial composition of environmental media and affects biodiversity and ecosystem services. (UNEP, 2022).

2.2 Search strategy

The search strategy was developed to be specific enough to be manageable within the time available, but also sensitive enough to identify the main evidence related to the review aim and questions. The search strategy was developed by an experienced information specialist, who identified key search terms from the two key sources below.

1. Review of reference lists supplied by ESS in the tender document and identification of relevant Scottish, UK, EU and international policies, government action plans and drivers.

2. Identification of key authors in the field by the scientific adviser on the research team along with key Scottish researchers identified on the Scottish One Health

AMR register (SOHAR) list (Holden *et al.*, 2021). Searches were carried out on authors' Google Scholar profiles and their publication lists on employers' online research repositories.

A MEDLINE bibliographic database search strategy was then formulated using controlled vocabulary (MeSH) and free-text terms. MEDLINE is the largest biomedical database available, with international coverage of over 5,000 current peer-reviewed biomedical journals (National Library of Medicine (US), 2023). To increase appropriate yield and ensure that relevant sources were not missed, the following databases were also searched:

- GreenFILE
- CAB Abstracts [all EBSCO]
- Web of Science [Clarivate]
- Agricultural & Environmental Science Database [Proquest].

The original search strategy used is shown in Table 1. The full search strategy applied in MEDLINE is presented in Appendix One.

Table 1. Original search strategy (Greenfile, EBSCOHost)

1. DE "ANTIBACTERIAL agents" OR DE "ANTIBIOTICS" OR TX ("anti biotic" OR antibiotic OR antibacterial OR "anti bacterial")
2. DE "Drug resistance in bacteria" OR DE "Drug resistance in microorganisms" OR TX ((microbial or bacterial) AND (drug resistance))
3. antimicrobial OR antimicrobial resist* OR AMR OR antibiotic resistant gene* OR ARG OR resistome OR mobilome
4. TI ("One Health" OR environment* OR water OR wastewater OR sewage OR river OR coast* OR aquaculture OR effluent OR sludge OR slurry OR animal OR cattle OR cow OR sheep OR pig OR poultry OR livestock OR wildlife OR food OR "food chain" OR soil OR compost OR air OR aerosol OR farm* OR crop OR plant)
5. 1 OR 2 OR 3

6. 4 AND 5
7. TI "meta analy*" OR metaanaly* OR review
8. 6 AND 7

6 AND 7 Limiters - Publication Date: 20170101-20231231

Google Scholar was also used for citation searching of key authors and to locate recent materials from the last year, which may not yet be recorded in academic databases. In addition, the websites of relevant organisations were searched for papers and reports relevant to the topic (Table 2).

Table 2. Websites included in the search

Scotland
Scottish Government
SEFARI (Scottish Environment, Food and Agriculture Research Institutions)
SEPA (Scottish Environment Protection Agency)
Environmental Protection Scotland
Marine Scotland Science
NatureScot
ARHAI (Antimicrobial Resistance and Healthcare Associated Infection) Scotland
Public Health Scotland
Food Standards Scotland
UK
UK Government
Environment Agency
OEP (Office for Environmental Protection),
Defra (Department of Environment, Food and Rural Affairs)
DHSC (Department of Health & Social Care)
APHA (Animal and Plant Health Agency)
VMD (Veterinary Medicines Directorate)
Food Standards Agency
UKWIR (UK Water Industry Research)

Welsh Government Animal and Environment Antimicrobial Resistance Delivery Group
Europe / International
Government of Ireland
WHO (World Health Organisation)
WOAH (World Organisation for Animal Health)
UNEP (United Nations Environment Programme)
FAO (Food and Agriculture Organization of the United Nations)
Organisation for Economic Co-operation and Development
European Parliament
European Commission
European Food Safety Authority

2.3 Search results

This comprehensive search returned 2,251 results, including both published and unpublished literature relating to AMR, its source, transmission and dissemination in the environment.

All search results were exported to Endnote 20 Reference Management software and a supplemental search was then carried out. This included referencing checking of already retrieved seminal papers for the identification of additional, relevant records. This produced a further 42 references after de-duplication. Scholar, MEDLINE, and GreenFILE were the most productive sources.

2.4 Inclusion criteria

As per our proposal, tailored and effective inclusion criteria were developed more iteratively than traditionally. This was done to ensure that priority was given to answering the review questions using the available literature within the given timeframe.

The final inclusion criteria were – date-based parameters; relevance to the Scottish focus of the review; and prioritisation criterion based on the quality of evidence (see Table 3) and geographic relevance.

1. If it was about the Scottish environment. Levels A through D. The oldest reference retrieved and deemed applicable to the review questions was published in 2007 (Nathwani and Christine, 2007).
2. Published between 2016 – Oct 2023; and meets criteria 3 or 4.
3. If it was Level A.
4. If it was level B AND relevant to Scotland (combination of quality and relevance).
5. Content was about high and upper middle-income countries (World Bank, 2023).
6. Published in the English language.

Table 3. Levels of evidence

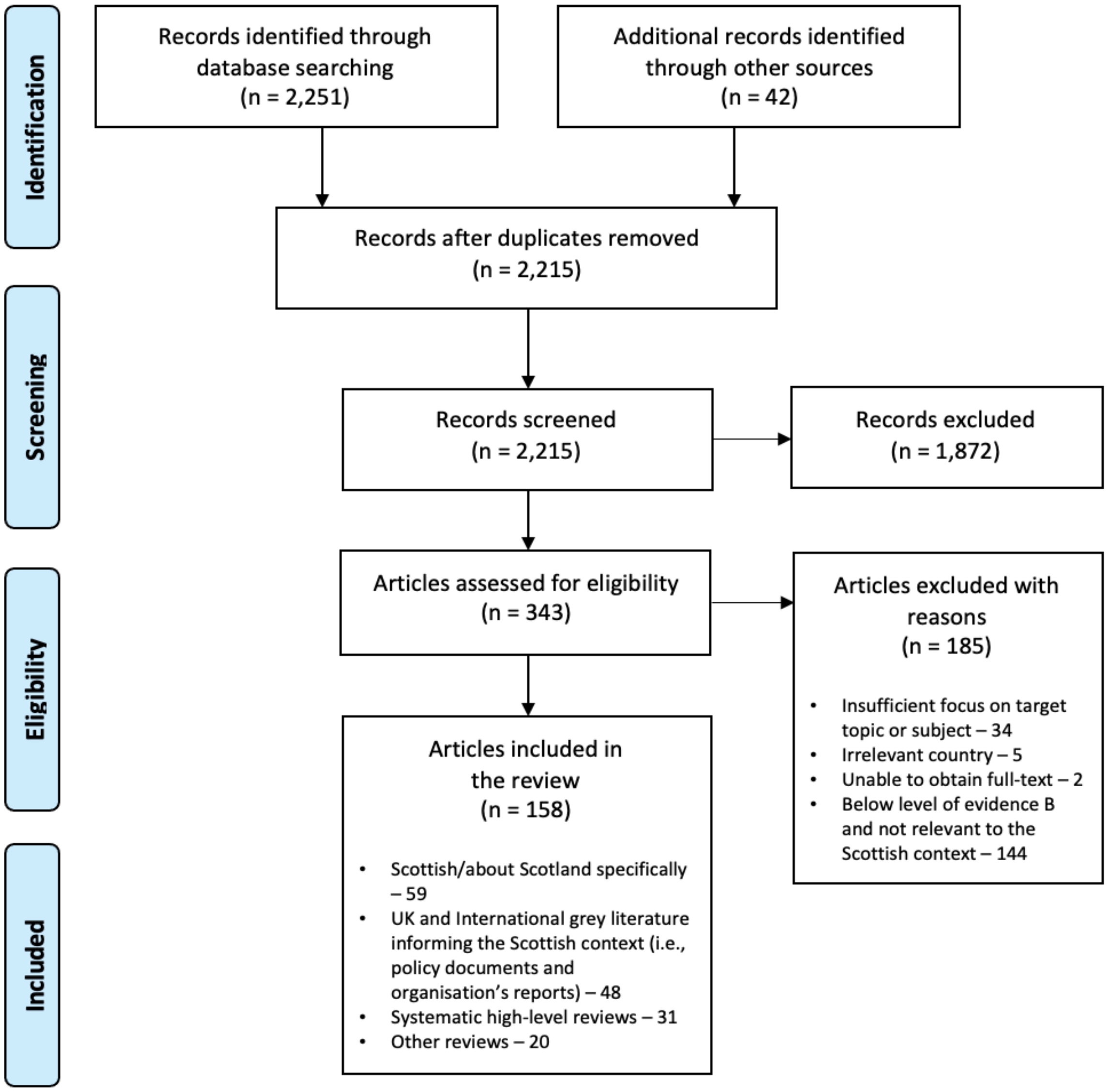
Level A	Meta-analysis / Systematic reviews
Level B	Scoping, Narrative and Rapid Reviews
Level C	Primary Research Studies
Level D	Grey literature – this included reports, policy/government documents, opinion articles, press releases, websites, blogs, conference posters and presentations

2.4.1 Evidence Screening & Data Extraction

After duplicates were removed, 2,215 items were screened by the title and available abstracts against the inclusion criteria in Endnote by two reviewers. The process resulted in 343 sources, which were subsequently extracted from Endnote into separate data extraction tables using Excel spreadsheets. A second screening process involved reviewing the content of each source remaining in more detail and resulted in 158 articles being included in the review. Of these, 59 were specifically about Scotland.

These Scottish-focused sources were used to establish the current status and understanding of AMR in the environment in Scotland. In addition, we included 31 systematic reviews, 20 other (scoping, narrative and rapid) reviews and 48 government reports/policy documents within the date range 2016 – 2023 from which relevant data was extracted and synthesised. These were included for relevance to AMR in Scotland with the synthesis of data that provided a richer information base to add to the information from the Scottish studies, and to enable comparison with neighbouring nations, including England, Wales and Ireland, and internationally. The outcome of the search and selection process is illustrated in Figure 1.

Figure 1. Flowchart of the study selection process



2.5 Strengths and limitations

The rapid nature of the review is associated with a key methodological limitation. Only high-level and secondary-level evidence was used to inform the review sub-questions not specifically about Scotland. Specifically, these are the questions involving the need to compare Scotland to the UK and international knowledge relevant to controls and impacts and the need to include possible forthcoming controls, which could be of relevance to Scotland.

The search strategy and inclusion criteria may also have resulted in a number of original research articles being excluded. However, the decisions made in relation to categorising the evidence ensured that the significance of the vast number of individual primary research studies, that are published across the globe every year on this topic, is not overstated by presenting their findings alongside those of systematic reviews and scoping, narrative and rapid reviews. As this was a time-limited exercise, no critical appraisal was undertaken of the sources gathered.

A key strength of this review is the wide-ranging grey literature search that was undertaken to ensure inclusion of all levels of Scotland-specific literature. A further methodological strength is the rigorous approach taken to screening and synthesising relevant literature, which was carried out by two researchers, first working independently and then comparing results. This process was overseen by the project's methodological expert and scientific advisor.

3. FINDINGS – AMR IN SCOTTISH ENVIRONMENT

3.1 Scottish landscape

In using the findings of this review to develop a better understanding of the resistance issue, its controls and impacts, in relation to the environment and how that applies to Scotland, it is important to first understand (i) Environmental Resistome and (ii) Scotland's distinctive landscape and the commercial and political framework in it. The environmental landscape of Scotland is diverse and still evolving in response to natural processes and the demands of society (Scotland's Environment, 2023). It consists of urban areas and managed countryside in Central and Eastern Scotland and less intensively managed uplands and coasts in Southern Scotland, the Highlands and the Islands.

3.1.1 Environmental Resistome

In terms of the scale and frequency of resistance genes and bacteria, the microbial communities present in all environmental compartments have been described as “wellsprings of resistance” (Surette and Wright, 2017). These resistance genes have developed within microbes in the environment for billions of years and collectively are known as the environmental or natural resistome (Surette and Wright, 2017; Larsson and Flach, 2022). Given the prevalence of this microbial pool across all environmental compartments, it is considered the largest persistent source of environmental resistance in terms of scale. The vast majority of resistant genes are present within microbes not known to be pathogenic and, therefore, pose no immediate AMR threat to health (Larsson and Flach, 2022). However, the potential for the evolution of new pathogens through the interaction of externally introduced microbes with these resident antibiotic-resistant genes (ARGs) is a concern regarding the evolution of AMR in the environment.

Improved understanding of the extent of the resistome is being aided by better access and affordability of sequencing technology, enabling the use of ‘metagenomics’ to study large volumes of bacterial genetic data. This technique was used in the first large-scale study of what has been termed the ‘latent resistome’ – the collection of ARGs that exist but have not yet been identified to have any clinical impact (Inda-Díaz *et al.*, 2023). The study concluded that the majority of these genes were associated with mobile genetic elements (MGEs), such as plasmids, and that several of these are already present in highly virulent human pathogens.

3.1.2 Scottish Agriculture & Aquaculture

Ninety-four percent of Scotland is classified as rural (Scotland’s Environment, 2023). Agriculture is the predominant land use, affecting nearly 80% of the land area (Scottish Government, 2023). Scotland’s climate, soils and topography influence the distribution of different farming systems across the country. The land, which can produce a variety of crops, is found along the East Coast, while most dairy production takes place in lowland grassland areas in the Southwest of Scotland (Scotland’s Environment, 2023).

Environmental Scotland (2023) report that a wide range of crops are grown in Scotland, and the country also produces livestock[2] and livestock products, with large areas of moorland used as rough grazing for sheep and cattle. The main pressures on the Scottish agricultural industry include the impacts of climate change, the need to protect the environment from the effects of agriculture, and changes in agricultural support payments (Scotland's Environment, 2023). Since 2005, there has been a decline in livestock numbers, predominantly in the uplands and islands.

Scotland has highly diverse farmlands, with a range of habitats and varying conditions. There has been a shift towards an intensive use of lowlands and farmlands for food production for people as well as livestock. However, this has led to a change in biodiversity that could have significant implications for food production due to a decline in pollinator populations (Scotland's Environment, 2023).

Aquaculture, the industrial farming of fish, has increased significantly in recent decades in response to feeding a growing population and addressing declining fish stocks (Watts *et al.*, 2017). The industry contributes £760 million annually to the Scottish economy, with produce representing 40% of Scotland's food production (Salmon Scotland, 2023). The industry is based predominantly within the rural West Coast Highland and Island areas of Scotland. It mainly includes the farming of salmon and sea trout held in sea pens; salmon and trout in freshwater pens and shellfish (oysters and mussels) on rafts and lines in the sea (SEPA, 2023b). The Scottish Environment Protection Agency (SEPA) protects the marine environment and is one of the agencies that regulate discharges from fish farms.

3.1.3 Scottish Wildlife

Scotland supports a diversity of wildlife and provides home to over 90,000 species (Scottish Wildlife Trust, 2023). There is an abundance of particular species of birds in Scotland, which indicates good biodiversity in the lowlands (Scotland's Environment, 2023), 2023). The main lowland species include butterflies, moths,

[2] **Livestock** – domesticated animals used for food (e.g., all farmed animals, including farmed fish and seafood animals)

birds, flowers, mosses and lichens. Britain’s heathland contains over 5,000 invertebrate species, alongside diverse wildlife, such as juniper and nesting stonechat. Other common habitats in Scotland’s lowlands and farmland include woodlands and wetlands.

The two key factors affecting wildlife in the Scottish ecosystem are changes in land use and land management practices, including applications of pesticides and herbicides (Scotland’s Environment, 2023). Almost two-thirds of habitats and species are either in recovering or favourable condition, with the remaining third in unfavourable condition. Several of the most threatened habitats are in the lowlands, notably lowland heath, neutral grasslands and fen meadows. Of 61 farmland bird species, nine significantly declined between 1995 and 2011, including kestrel, oystercatcher, lapwing, swift, rook, skylark, starling and meadow pipit (Scotland’s Environment, 2023).

The current key pressures on Scottish wildlife across the following habitats include (but are not limited to):

- Uplands – high levels of deer and sheep grazing together with drainage of peatlands and atmospheric pollution of nutrients and harmful compounds (e.g., nitrogen and sulphur oxides emitted from fossil fuel burning and vehicles).
- Grasslands – there are fragmented habitats in the lowlands, with only about 1% considered wildlife-rich. Most pressures that adversely affect wildlife come from fertilisers, herbicides, pesticides and drainage and reseeding of grasslands.
- Freshwaters and wetlands – rivers have been affected by changes in water flow and quality. These habitats have deteriorated as a result of sewage discharges, and diffuse pollution from run-off of soil, nutrients and pesticides from farmland and forestry.
- Coast and seas – the two key pressures on the marine environment include commercial fisheries and localised pollution from aquaculture (Scottish Wildlife Trust, 2023).

The Scottish Government has been actively working with partners to manage the wildlife and to protect the country's natural resources by conserving rare and vulnerable species.

3.1.4 Scotland's Water Resources

Scottish rivers and lochs cover about 2% of the country's land area but they contain 90% of the UK's surface freshwater (Scotland's Environment, 2014). Water in Scotland is used for drinking and also in industry (e.g., distilling whisky and supporting fisheries), for producing energy (hydropower), and for recreational activities (e.g., birdwatching, angling and water sports). There is an array of habitats in Scottish waters, which contains important populations of some species.

Scotland receives an abundance of rain, which collects in lochs and rivers. In many places, it is collected behind dams in reservoirs. Scottish Water provides the majority of the public drinking water and sewerage services in the country (Scottish Water, 2023b). This is a public company accountable to Scottish Ministers and the Scottish Parliament, which is responsible for treating water and ensuring it is free from chemicals and bacteria. The supply of private water in some parts of Scotland is mostly the responsibility of owners and users and is regulated by local authorities.

The condition of Scottish seas, coasts and estuaries has been reported to be in good or excellent condition, with significant reductions in pollution over the last 25 years (Scotland's Environment, 2014). The Scottish Government plays an active role in the protection and improvement of all fresh and marine waters in Scotland, including rivers, lochs, estuaries, coastal waters and groundwater through River Basin Management Plans (RBMPs), produced every six years. These improvements are reached through programmes of work carried out by SEPA and delivered with a wide range of partners (Scotland's Environment, 2014).

3.2 Causes of AMR in the environment

This section provides an overview of the literature on the causes and pathways of AMR genes and AMR-carrying bacteria into the environment. The term AMR (antimicrobial resistance) includes resistance by microorganisms (bacterial, viral, fungal and parasitic organisms) to all chemicals with antimicrobial abilities. In many studies, AMR is often used interchangeably with the term antibiotic resistance (resistance of bacterial species to antibiotic drugs). We first describe the literature pertinent to Scotland and then, the UK and international literature of relevance.

3.2.1 Cause: Human Waste

Human waste is considered a significant cause, or pathway, of entry of AMR into the environment. In addition to the excretion of antibiotic-resistant bacteria (ARB) and ARGs, human waste is also rich in antimicrobials. An estimated 30-100% of orally administered drugs leave the body unmetabolised and enter wastewater (Pagaling, Troldborg and Zhang, 2023). Scotland's Centre for Expertise in Water (CREW) have undertaken an assessment of pharmaceuticals in the water environment in Scotland and identified a total of eight substances as posing a higher risk in Scottish waters (Helwig *et al.*, 2022).

3.2.2 Cause: Wastewater Treatment Plants (WWTPs)

Four Scottish studies have investigated AMR in human wastewater, three of which used metagenomics which identify the full range of ARGs and one used qPCR which is more limited in its range (Perry *et al.*, 2019, Perry *et al.*, 2021 and Lepper *et al.*, 2023). A comparison of wastewater from tertiary hospital and a community sewage works found that the overall ARG abundance was higher in hospital wastewater than in community influent. Although total antimicrobial usage was not associated with higher ARG abundance in wastewater, there was a positive association between resistance to the antibiotics carbapenem and vancomycin and increased usage. Prolonged admission was found to be a risk factor for carriage and infection with resistant microorganisms. Perry *et al.* (2019, 2021) concluded that antimicrobial usage is a major driver of AMR from Scottish hospitals into the sewage environment.

Although still requiring further evaluation, the authors suggest that metagenomics may represent a useful surveillance tool to monitor hospital AMR and guide environmental policy on AMR.

Lepper *et al.*, 2023 compared the resistome and microbiome of hospital, community, and mixed municipal wastewater using metagenomics. Eight WWTPs, four hospitals, and four community sites in Scotland were sampled. The study found that hospital and community wastewater resistomes differ, with the hospital wastewater representing a reservoir of patient and hospital environment-associated bacteria. This supports other studies, which report that hospitals act as a reservoir and enricher of resistance. Knight, 2022 used qPCR (quantitative polymerase chain reaction) to screen wastewater, the receiving marine environment and the River Dee in Aberdeenshire for seven ARGs and the class 1 integron gene (a proxy for AMR). ARGs were detected in each of these samples. Of relevance is also a large UK study of AMR, which found extended-spectrum beta-lactamase (ESBL) producing *E. coli* in several Scottish sewage samples. However, no data on abundance or prevalence was provided in the paper (Day *et al.*, 2019). One other study found that plastic waste within the Firth of Forth in Scotland acted as a reservoir for faecal bacteria (Metcalf *et al.*, 2022).

A national review of AMR evidence carried out in Ireland in 2021 (Cahill *et al.*, 2021) noted that human waste has been highlighted by several empirical studies as a significant source of AMR in the environment of Ireland. The authors report that multi-drug resistant organisms have been found in the Irish aquatic environment and identify human and animal waste streams as the most likely sources. Findings from work undertaken in Ireland include the detection of a high level of resistance to penicillins within WWTP effluent as well as resistance antimicrobials called cephalosporins.

Two Irish literature reviews conclude that the current WWTP processes are not capable of removing all ARB/ARGs (Cahill *et al.*, 2021; Monahan *et al.*, 2022). These authors further note particular concern in the finding of persistence of *E. coli* resistant to a wide range of antimicrobials, including ampicillin, streptomycin, cefoxitin, cefotaxime, tetracycline, sulphonamide and ciprofloxacin following treatment, as such contaminants are subsequently released into the environment (Cahill *et al.*, 2021). Overall, the majority of the studies reviewed highlighted that wastewater (including hospital, municipal and WWTP effluents) is a major source of AMR in the environment in Ireland (Cahill *et al.*, 2021; Monahan *et al.*, 2022).

3.2.3 Cause: Private Wastewater Systems and Septic Tanks

Private wastewater systems are another source of AMR into the Scottish environment. In remote and rural areas not connected to main sewer systems, household water waste is deposited into septic tanks. Waste is broken down by bacterial processes within the tank and subsequently drains to either a closed soakaway surface or ground water. SEPA (2019) estimate that there are 250,000 private wastewater systems in Scotland, a significant proportion of which discharge to land via a drainage field. The environmental performance of these wastewater systems is significantly poorer than those operated by the public sector (SEPA, 2019).

A requirement to upgrade septic tanks in England by 2020 did not apply in Scotland (SEPA, 2023a). To effectively protect the environment, SEPA indicate that an appropriate design, inspection, maintenance and upgrade of private wastewater systems is required (SEPA, 2023a).

3.2.4 Cause: Combined Sewer Overflows (CSOs)

Combined sewer overflows (CSOs) are used during times of heavy rainfall to remove excess flow in the system and prevent backfill to homes and businesses. Their use leads to the disposal of untreated sewage into rivers and waterways. In Scotland, there are 3,614 CSOs within 50,000km of the sewer network (equivalent to one for every 15km), operated by Scottish Water and regulated by SEPA (Scottish Water, 2023a). Around 4% of overflows are monitored and their overflow spills are reported to SEPA annually. In their 2022 review, Stanton *et al.* note that the impact of CSOs on AMR selection is understudied and that it is an area that has become of public, policy and scientific interest.

Singer *et al.* (2021) review of international evidence further reports that it is difficult to determine true CSO discharge to freshwater as these areas are under-monitored and data is often not made publicly available by water providers. The authors point out that data often reports the length of discharge time but not volume. As an example of the scale of the issue, the report highlights that in 2018, an equivalent of 39 years of untreated sewage was dispensed into Welsh rivers (Singer *et al.*, 2021).

England's Environment Agency (2022a) used the presence of CSOs and their frequency of use as selection criteria for the identification of suitable AMR surveillance sites. The authors highlight that sewage discharge is likely to dominate the AMR signal found in freshwaters. This assumption has also been made in a Welsh government report by Singer *et al*. (2021). They explain that untreated sewage, such as that released from CSOs, will contain a significantly higher load of AMR genes, antimicrobials and total microorganisms in comparison to treated wastewater by approximately two to three orders of magnitude (Environment Agency, 2022a).

3.2.5 Cause: Spreading of Sewage Sludge on Land

The use of WWTP sewage sludge (also known as biosolids) as a fertiliser for agricultural land is another potential route for AMR entry in Scotland. SEPA (2023a) report that over 70% of sludge produced in Scotland is used in this way. Biosolids, the solid end-product of wastewater treatment, are rich in nitrates, phosphates and other organic material. In the UK, it is legal for this sludge to be used as a fertiliser on farmland although this is strictly regulated by the Sludge Regulations 1989. The UK Government have set standards related to sludge treatment processes (including biological, chemical, heat treatment and long-term storage), which are required to minimise or eliminate the presence of pathogens (Environment Agency, 2018).

In Scotland, there are six different types of sludge treatments, which are divided between sites that are owned and operated by a) Scottish Water (including 32 'core sites'), and b) third parties (21 Scottish Water Private Public Partnerships (PPP)), with the majority of sludges (80%) destined for use in agriculture produced by PPP sites (Scottish Government, 2021). Before sewage sludge can be spread on land, an operator must first register with SEPA and follow their regulations (e.g., the sludge must be stored not less than 10 metres from any inland or coastal waters) (SEPA, 2020). However, evidence shows that treated sludge contains a range of pathogens. In 2021, a study in Scotland reviewed the human health risk of AMR in sewage sludge (Hough *et al.*, 2021a). This study reported the presence of multiple drug resistant *Klebsiella pneumoniae* in sludge. The authors highlight that the dangers of AMR within sludge remain unclear.

Drinking water treatment also generates solid, organic sludge waste. This sludge makes up around 25% of the combined total sludge and about 99% of this sludge is re-used in land reclamation. The AMR content of Scottish water treatment works sludge is unknown.

3.2.6 Cause: Agriculture / Aquaculture

The direct application of livestock waste (manure or slurry) to agricultural land and subsequent run-off into surface water is also considered a significant introductory route for AMR (UKHSA, 2022). The agriculture and aquaculture sectors are users of antibiotics, and hence also introduce antimicrobials and other AMR-selective chemicals to the environment. Agriculture is a key industry for Scotland with over 80% of Scotland's land used for agriculture (Scottish Government, 2023). The aquaculture sector is also significant, with Scotland being the third biggest producer of farmed Atlantic salmon in the world, after Chile and Norway (Scottish Government, 2016).

Agriculture

In Scotland, *E. coli* in the faeces of healthy livestock has been monitored for AMR annually since 2017 (SRUC, 2023). AMR levels are reported to have remained generally low for cattle and sheep, but in contrast, were consistently higher for pigs and poultry. The low AMR levels in sheep were also found in a small study of healthy

Scottish sheep sampled from abattoirs and field flocks (Tongue *et al.*, 2018). In contrast, in a study of 108 Scottish cattle herds, a high herd-level prevalence (77%) was estimated for the class I integron, recognised as a proxy for AMR. They also observed a regional difference with the highest prevalence in the Northeast and Southeast and the lowest in the Highlands (Fernández Rivas *et al.*, 2021).

From the high-quality evidence reviewed, a total of seven systematic reviews specifically assessed the prevalence of AMR in agriculture and their findings are of relevance. Foyle *et al.* (2023) looked at antimicrobial resistance in effluent wastewater from animal slaughter facilities. Two studies focused on very specific bacteria, including *Aeromonas* (Jones *et al.*, 2023) and Carbapenem-resistant *Enterobacteriaceae* (CRE) (Köck *et al.*, 2018). They found that resistance to all major antimicrobial classes, (ceftazidime, piperacillin, gentamicin, ciprofloxacin, and chloramphenicol) is variable.

The data indicate that human activities in farming systems are a major contributor to the cause and dissemination of AMR into the environment via slaughterhouse effluents (Foyle *et al.,* 2023). Jones *et al.* (2023) reviewed data from several sectors, including human, agriculture, aquaculture, drinking water, surface water, and wastewater samples, and found minimal differences between sectors among 21 different antimicrobials. Whilst Köck *et al.* (2018) reported the occurrence of CRE in livestock, seafood, wildlife (up to 19% of carried CRE), pets, and in directly exposed humans.

Furthermore, Wu *et al.* (2023) reported on sources of antibiotics and ARGs in agricultural soils. The main antibiotic pollutants were found to be sulfonamides, tetracyclines and fluoroquinolones, with corresponding high levels of associated ARGs. Several factors (land-use type, soil, and climatic factors) affected antibiotic concentration and ARG abundances. In addition, mobile genetic elements (MGEs) were found to play a major role in promoting the dissemination of ARGs, especially genes associated with the integron 1 gene.

Evidence for increases of ARG in the natural environment associated with potential sources of ARB and ARGs was the focus of the Bueno *et al.* (2017) systematic review. In the context of the very diverse evidence base identified, the authors found that most of the studies reviewed reported higher ARG concentration downstream/near the source, such as agricultural facilities and wastewater treatment plants.

A Chinese meta-analysis of 94 published studies investigated the impacts of farmland application of antibiotic-contaminated manures on antibiotic concentrations and ARG abundances in manure-amended soil (Zhang *et al.*, 2022). They report that ARG “abundances” could be correlated with soil silt content, antibiotic concentrations and mean annual temperature, mobile genetic elements and soil pH ($P < 0.05$).

Aquaculture

Farmed fish populations are highly susceptible to a variety of infectious agents – bacterial, viral and parasitic that, once established, are easily spread amongst the population and cause significant economic loss. The use of antimicrobials to treat infections in farmed fish is tightly regulated in Europe, with a ban on the prophylactic use of antibiotics in 2001. Vaccinations have been successfully developed as an alternative. For example, Norway reduced their antibiotic use by 99% between 1987 and 2013, alongside a 20% increase in fish production (Scottish Government, 2016). In UK, all salmon farms are based in Scotland. Data collation and overall antibiotic stewardship in Scotland is the responsibility of the Salmon Scotland Prescribing Vets (SSPV) group.

The 2021 VARSS report, published by the Veterinary Medicines Directorate (VMD, 2022), showed that antibiotic usage in salmon aquaculture was the second highest among the UK food-producing animal species (2021 data – salmon usage 43mg/kg and pig usage 87mg/kg). This represented an increase of 168% in usage between 2017 and 2021 and was the only increase among food animal species reported. The more recent data represent a decrease in the salmon usage in 2022, however, it still remains 15% above 2017 usage (VMD, 2023). Salmon Scotland (2023) point out that antibiotic treatments are not common in the sector, with only 1.5% of freshwater farms and 8.7% of marine farms treated in 2022 (VMD, 2023). They further report an increase in UK antibiotic usage in trout farms by 130% between 2021 and 2022. This was attributed to an outbreak of *Aeromonas salmonicida* on a small number of production sites (VMD, 2023). It is not clear from the report how many of these trout farms are in Scotland. In their latest 2022 VARSS report, the VMD (2023) explain that "this sector is particularly prone to fluctuations in its yearly antimicrobial use trends as a small number of disease outbreaks can have a large impact on the individual sector's usage".

3.3 Impact of AMR in the Environment

The environment functions as a reservoir, where AMR accumulates and is disseminated across a landscape (WHO, 2022). The environment also functions as the substrate for ecological processes which drive the evolution of organisms.

3.3.1 Impact: Health of the Environment

The Quadripartite Organizations, made up of the Food and Agriculture Organization of the United Nations (FAO), United Nations Environment Programme (UNEP), World Health Organization (WHO), and the World Organisation for Animal Health (WOAH), have defined the health of the environment as "the extent to which the environment is able to function, maintain biological and chemical processes, adapt to change or cope with the impacts of human activity". AMR presents a risk to the health of people and animals by making antimicrobials ineffective. However, it is not clear how, or whether, the acquired ability to survive antimicrobial exposure (resistance) could impact the health of the environment. Larsson *et al.* (2023) warn against confusing the direct ecological effects of antimicrobial pollution with the effects of AMR. They further explain that carrying ARGs may enhance the colonisation ability of AMR organisms, as they will be able to survive when antimicrobial pollution is present. Importantly, a resistant organism in the environment only becomes a problem if it causes infection in humans, animals or crops (Larsson *et al.*, 2023).

However, there is some evidence that AMR selective chemicals in the environment may cause adverse effects directly on the health of the environment. Davison, Macadam and Smith (2021) have reviewed the potential effects on freshwater invertebrates of various pharmaceuticals, including antibiotics. They include analysis of data from the Chemical Investigations Programme 2 (CIP2), which took place in England, Wales and Scotland between 2015 and 2020 (UKWIR, 2023). The CIP and CIP2 (Scotland) findings reveal that many pharmaceuticals identified in the freshwater environment are present at potentially dangerous levels. All 14 substances in the study at times exceeded their Predicted No Effect Concentration (PNEC) or Ecological Quality Standard (EQS).

Intense periods of elevated concentration levels can impact on the growth, behaviour and reproduction of freshwater invertebrates. Although unknown as yet, there is the potential that this will further impact on birds and fish by reducing their diet source or exposing them to contamination by eating invertebrates or insects (Davison, Macadam and Smith, 2021). The authors highlight that Wastewater Treatment Works play a key role in contributing to these levels. They also note that "very little literature exists on the effects of antibiotics on invertebrates in general, especially for freshwater invertebrates", and that "the studies that do exist all use concentrations well above what is found naturally in the environment". The CIP3 programme plans to analyse other sources of pharmaceuticals, such as biosolids and septic tanks and encourages examination of other sources, such as sewer overflows, river and pond sediments, landfill run-off, and agricultural run-off and to undertake long-term examinations of effects on invertebrates in the environment.

A review by Danner *et al.* (2019) reported antibiotic concentrations in surface fresh waters on different continents (Americas, up to 15 µg/L, Europe over 10 µg/L, African studies over 50 µg/L, Asian-pacific countries over 450 µg/L). Laboratory experiments have shown that such sub-inhibitory concentration may change the composition of single-celled communities. This will, in turn, have implications for the microbial food web (e.g., interactions among and between bacteria and their protozoan consumers). By extension, they suggest that larger organisms and ecosystem health will be affected.

3.3.2 Impact: Transmission of AMR to Humans, Animals & Crops

The transmission of resistant bacteria and genes between humans, animals and the environment is understood to occur commonly (Larsson and Flach (2022).

A recent systematic review found 40 studies that provided empirical evidence of health outcomes in humans associated with exposure to antibiotic resistance in the natural environments (Stanton *et al.*, 2022). Four exposure routes were investigated, including consumption/ingestion, inhalation and direct contact, with the most studied route being consumption/ingestion. The health outcomes assessed were exposure, infection, colonisation and mortality. Only two studies reported on the latter, with the majority focusing on colonisation associated with exposure to aquatic environments (Stanton *et al.*, 2022). While our review did not identify any Scottish evidence on this, both Larsson and Flach (2022) and Manaia *et al.* (2022) reviews highlighted that health outcomes are difficult to quantify and find direct evidence on.

A review by Manaia *et al.* (2022) concluded that despite widespread reports of clinically relevant ARB and ARGs in the environment, it has been difficult to date to find clear evidence of direct transmission to humans. There is often limited evidence available to trace human health outcomes, such as infection and mortality, back to the natural environment. Individuals may be colonised for long periods of time before an infection occurs. Traceback is equally challenging if the infection results from onward transmission from a colonised individual to a vulnerable contact (Manaia *et al.*, 2022).

Risk of Transmission via Water

In 2022, CREW published a Policy Brief and Policy note, reporting on a project, which evaluated the current status of AMR in Scotland’s waters (Bridle, Pagaling and Avery, 2022). Their findings highlighted a need for more consistent approaches to water monitoring, for example, to test isolates for extended-spectrum beta-lactamase production (ESBL), which confers resistance to antibiotics, such as penicillins and cephalosporins, also for an agreement on the key resistance genes to target through molecular methods. The authors 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 (Bridle, Pagaling and Avery, 2022). They also recommend that consistency in the chosen methods can be further developed through consideration of sampling (volumes, handling, storage), protocols (standard operating procedures, researcher training), and data processing (analysis, interpretation, visualisation, management, storage, quality assurance and sharing).

Another review looking at the potential impact of resistant bacteria in bathing water on human health suggests that ingestion of environmental bacteria by surfers and bathers could lead to ARGs being acquired by commensal and opportunistic pathogens in the gut microbiome (Leonard *et al.*, 2022). It concludes that frequent exposure to resistant *E. coli* is likely among various water users studied but is particularly high among surfers and children, who tend to ingest larger volumes of water.

SEPA has undertaken surveillance of *E. coli* pathogen resistant to an antibiotic called cefotaxime (CTX-R) in water samples collected from Scotland's designated bathing water sites during the bathing water season (June to mid-September) from 2018 to 2023 (except 2020) (ARHAI Scotland, 2022). CTX-R *E. coli* were detected in 58-78% of bathing waters, with the proportion of CTX-R *E. coli* ranging between 0.25% to 0.82%. In 2022, SEPA added the monitoring of the Enterococcus pathogen resistant to antibiotic vancomycin (VRE). This was in response to the high level (40%) of VRE in clinical samples in Scotland, which was much higher than in England and one of the highest in Europe (ARHAI Scotland, 2022). Subsequently, SEPA make developed an online dashboard to present and share this data, (SEPA, 2023c).

Khan, Knapp and Beattie (2016) investigated the diversity of microorganisms in tap water in Glasgow in Scotland. A number of different bacterial species were isolated and some of these carried ARGs. Biofilms (structured microbial communities that occur as surface-attached communities or suspended aggregates) in the distribution system or plumbing system are a recognised factor contributing to microorganisms in the drinking water system. In a related study, further analysis of tap water in Glasgow found that bacteria that survive chlorine treatment are more likely to also be resistant to antibiotics (Khan, Beattie and Knapp, 2016).

Private water supplies (PWS) and the associated septic tanks provide another potential pathway for transmission of AMR. In 2018, there were 21,980 known PWS in Scotland, serving 196,536 people (3.6% population). The actual number of users may be higher as these figures do not always account for visitors (Teedon *et al.*, 2020). These private supplies are reported to have a higher level of faecal indicator failures than public supplies and in this way, represent an increased risk of AMR to humans, via transmission from the environment. This risk is increased further from the proportion that discharge to land by way of a drainage field (SEPA, 2019).

Of relevance, an Irish study noted a correlation between a carbapenemase producing *E. coli* in environmental and clinical isolates. An NDM-producing *E. coli* (a protein that certain bacteria produce) detected in one of the freshwater streams and the wastewater system were identical to a clinical isolate detected in Ireland previously (Hooban *et al.*, 2020). Co-occurrence of antibiotic resistance in the environment and humans is regarded as poor direct evidence of transmission of resistance from the environment to humans (Stanton *et al.,* 2022). However, when the strain in both human and the environment are identical by molecular typing methods, such as cgMLST (core-genome multilocus sequence typing), the evidence is stronger (Hooban *et al.,* 2021).

The gap analysis undertaken in Ireland has shown that the high levels of faecal contamination and ARGs in the river estuary were most likely due to effluent discharged from the nearby WWTP (Cahill *et al.*, 2021). However, as the urban streams and bathing waters did not receive any known wastewater discharges, sewer overflows and pipe leaks were suggested as potential sources of contamination. Findings further indicate that faecal and ARG contamination of the aquatic environment in Ireland may be fuelled by gull and dog waste. However, the authors emphasise that further research on their overall contribution to AMR contamination is required (Cahill *et al.*, 2021).

In addition to surface waters, groundwaters were identified as potential reservoirs of AMR (Andrade *et al.*, 2020). In Ireland, overall, *E. coli* resistant to both human and veterinary antimicrobials were detected in groundwater wells (O'Dwyer *et al.*, 2017). Causative factors included links between the presence of young children in households and septic tanks and the *E. coli* detected in groundwaters, which were resistant to antimicrobials used in human medicine, including penicillins, cephalosporins, fluoroquinolones, nitrofurantoin and trimethoprim. This finding is concerning due to the potential risk the resistant pathogens may pose to human health, especially as these waters may serve as drinking water sources. Monitoring of groundwater is important in Scotland as it provides 73% of drinking water for private water supplies (supplying at least 80,000 people), and 5% of Scotland's public water supply. Groundwater is also used for all the bottled water industry (14.7 million litres per day) and for 70% of water bottled by the distilling industry process. In addition, it is extracted for a variety of other industries including fish farming, mining, agricultural irrigation, and brewing (Scotland's Environment, 2011).

One global review reported water as the environmental compartment most investigated for AMR bacteria acquisition, diversity, and the interspecies spread between humans, animals and the environment (Meier *et al.,* 2022). The key conclusion from this research was that available evidence provides a "fragmented and incomplete understanding of AMR acquisition, diversity, and the interspecies spread between humans, animals and the environment".

A review looked at the potential impact of resistant bacteria in bathing water on human health. It suggested that ingestion of environmental bacteria by surfers and bathers could lead to ARGs being acquired by commensal and opportunistic pathogens in the gut microbiome (Leonard *et al.*, 2022). It concludes that frequent exposure to resistant *E. coli* is likely among various water users studied but is particularly high among surfers and children, who tend to ingest larger volumes of water.

Risk of transmission via Food

Ingestion of food that contains AMR is considered a potential risk route for humans. We identified only one study in Scotland that has investigated this risk.

In a Scottish study carried out by Bishop *et al.* (2022), a baseline survey was undertaken of AMR in 1,009 fresh minced beef samples from 15 geographic areas, including five categories of retail outlets in Scotland from January to December 2019. A low prevalence of antimicrobial resistance was found, but the authors commented that this did not give cause for concern as they occurred mainly in a few *E. coli* isolates. However, they suggested that routine monitoring should be implemented to mitigate any public health risk.

Day *et al.* (2019) investigated the contribution of the food chain to infections with ESBL-*E. coli*, a common food-associated pathogen which causes more than 5,000 cases of infection annually in the UK. The aim was to identify reservoirs of ESBL-*E. coli* that colonise and infect humans to identify strategic intervention points. They tested human faeces, sewage, farm slurry, and retail foodstuffs in London, East Anglia, northwest England, Wales, and Scotland between Aug 2013 and Dec 2014. The study found that 11% of human faeces samples contained ESBL-*E. coli* and were also frequent in sewage. A high percentage (65%) of the 159 retail chickens sampled were also positive for ESBL-*E. coli*. However, they were rare in other meats and absent from plant-based foods (0 of 400 fruit and vegetable samples) (Randall *et al.*, 2017). This latter result is a significant finding as fresh produce are considered a likely route of transmission of AMR from manure or biosolids-treated soils.

Another significant finding from the Day *et al.* (2019) study was that the strains that predominated in human-related sources (blood, faeces and sewage) were different from those which predominated in food and veterinary isolates (farm slurry, and retail foodstuffs). This is similar to the findings of Mather *et al.* (2012), who compared long-term surveillance data of AMR phenotypes in *Salmonella Typhimurium* DT104 isolates from human and animal populations in Scotland. Mather, *et al.* (2012)

concluded that, while ecologically connected, animals and humans have distinguishable DT104 communities, differing in prevalence, linkage and diversity.

However, Day *et al.* (2019) found that one strain of *E. coli* (ST10) occurred in both human and animal samples, though the serotypes in these two groups of ST10 were diverse. A similar finding was reported in a preliminary Food Safety Scotland pilot study from PATH-SAFE initiative, which sampled a large number of animal (livestock, companion[3] animals (dogs) and wildlife (deer) and human infection samples (FSA, 2022). While a large diversity of strains (ST types) was found in the human and animal samples, the ST10 strain was found in all the animals and in the human samples.

International studies have also reported contamination of food sources with AMR bacteria. For example, a meta-analysis of surveillance of AMR in food sources from China and the USA concluded that of over 13,000 food samples taken, 5,000 (over 38%) were found to be contaminated (Himanshu *et al.*, 2022). A South American prevalence study reported 'alarming levels' of AMR of common food-transmitted bacteria in Argentina (Prack McCormick *et al.,* 2023). A systematic review that included 256 studies, found that eight studies (18%) suggested evidence of transmission of AMR from food animals to humans, 25 studies (56%) suggested transmission between animals and humans, with no direction specified, and 12 studies (26%) did not support transmission (Muloi *et al.*, 2018).

Focusing on food crops, a meta-analysis was conducted to determine the prevalence of resistance to antibiotics, such as tetracycline (TET) and third generation cephalosporins (3GC) in bacteria called Enterobacteriaceae, isolated from food crops (Brunn *et al.*, 2022). TET is a potent broad-spectrum antibiotic widely used in agriculture and there have been increasing concerns about its resistance to 3GC in the environment. 3GC resistance was found to be highest in Africa and lowest in Europe. The authors consider the results of this work to support the inclusion of 3GC

[3] **Companion animals** – domesticated animals used for company as pets (e.g., dogs, cats, horses, etc)

in AMR surveillance in food crops and that food crops should be included in One Health AMR surveillance.

Risk of transmission via Soil & Air

The development of a Scottish nationwide map of resistance gene abundances in rural soils shows that while some forms of resistance are an endemic part of microbial ecology in soils, others are localised. The work identified that genetic diversity is driven by environmental factors (e.g., heavy metals) and agricultural practices (Akoumianaki *et al.*, 2020).

Knapp *et al.* (2011) quantified the abundance of eleven ARG and compared their levels with geochemical conditions in randomly selected soils from a Scottish archive. Certain heavy metals may serve as a selective agent for antibiotic resistance. This study investigated the relationship between metal levels in nature and the intrinsic presence of ARG. It found ARG correlated with soil copper, chromium, nickel, lead, and iron levels and suggested that soil geochemical data might be used to estimate baseline ARG presence. Rodgers *et al.* (2019) have reviewed the legacy of industrial pollution in estuarine sediments and highlight that in areas of history of high industrial activity, such as the River Clyde in Scotland, pollutants persist which could influence antimicrobial resistance.

Knapp *et al.* (2019) have developed a large dataset, which collates the relative concentration of nearly 300 AMR genes found in soils, sampled 2007-2010 at 183 soil locations across all of Scotland. Knight, 2022 used a microcosm experiment with four distinct Scottish soils amended with pig manure to investigate the relative abundance of ARG called sul1, which has been identified as a widespread contaminant in soils. Sterile soils were amended on day 0 and tested for ARG over 100 days. The pig manure amended soils showed a significant increase in sul1 ARG between days 1 and 7. Following this peak, the gene was observed to steadily decline. However, even by day 100, the gene remained at a significantly higher level compared to that of the unsterilised soil.

Air is another potential environmental medium of AMR transmission, particularly for respiratory pathogens. This route is particularly important for transmission of antimicrobial resistant fungi (AFR) from the environment to humans (Environment Agency, 2022c). A systematic review by Ginn, Lowry and Brown (2022), which reviewed studies investigating AMR in air, found that AMR enteric microbes have been consistently reported in outdoor aerosols, though gaps remain, preventing full understanding of the role of the aeromicrobiological pathway in the fate and transport of enteric associated outdoor aerosols.

England's Environment Agency has recently developed a sampling strategy and assessment options for environmental antimicrobial resistance in airborne microorganisms (Environment Agency, 2022b). As demonstrated for COVID-19, airborne pathogens can transmit very quickly and cover vast geographic areas. In a separate report, they express concern at the lack of concerted UK research on airborne AMR, suggesting that any potential risk through this route remains unknown (Environment Agency, 2020).

Risk of transmission via Wildlife

We identified two Scottish studies that report the potential for wildlife to act as reservoirs for AMR. A Scottish Masters' thesis published in 2021 identified resistant bacterial populations in wild birds, suggesting that they could serve as a reservoir for AMR (Djuwanto, 2021). The study measured extended-spectrum β-lactam (ESBL) resistant coliforms in gulls and geese populations across rural and urban Scotland. It reported that gulls in urban areas, near WWTPs, have significantly higher levels of ESBL-*E. coli* than in rural areas (57% urban vs 2% rural). These gulls also carried some multi-drug resistant *E. coli*.

Another recent Scottish study of relevance tested for AMR *E. coli* in the faeces of wild deer (Elsby *et al.*, 2022). Resistance to clinically important antimicrobials, such as carbapenems and third generation cephalosporins was found to be low. In a follow-up publication (2022), the authors reported that the prevalence of resistant *E. coli* was 21.8% for antibiotic tetracycline, 6.5% for cefpodoxime, 0.3% for ciprofloxacin, with no recorded resistance to meropenem. Distance from wastewater treatment plants was a significant risk factor for tetracycline resistance in *E. coli* from red deer, but not from roe deer. While this is an individual study and further research is required, the data indicate that AMR *E. coli* can occur in wild deer populations that are not directly exposed to the selective pressure exerted by antimicrobial treatment. However, they conclude that overall, resistance to critically important antimicrobials is low and suggest that there is no immediate cause of concern to human health. A Scottish Group is undertaking a survey to determine whether seals carry AMR genes and to characterise antibiotic-resistant *Campylobacter* (Bestwick, 2022).

A scoping review on the role of wildlife in the transmission of bacterial pathogens and AMR to the food chain by Greig *et al.*, (2015) reported that an association between wildlife and transmission of bacterial pathogens and/or AMR to the food chain was supported in 122 studies, particularly the transmission of *E. coli*, Salmonella and Campylobacter. Most studies investigated birds, cervids, rodents, feral pigs and opossums. This highlights the need for continued monitoring and assessment of this risk in Scotland, particularly around migratory bird populations.

In 2022, an international review highlighted the importance of wildlife as sources/reservoirs, pathways and receptors of ARB/ARGs in the environment, thus paving the way for future primary research in these areas. They report on the detection of resistant forms of S. aureus (MRSA), *E. coli*, *K. pneumoniae*, *E. faecalis* and *E. faecium*. They highlight that antimicrobial resistance among urban and migratory animals represents a particular concern (Abbassi *et al.*, 2022).

3.3.3 Impact: Evolution of AMR

Antimicrobial resistance is an evolutionary process where microorganisms evolve to become resistant to antimicrobial treatments, to which they were previously susceptible (Larsson and Flach, 2022). This evolution occurs in response to the presence of AMR selective chemicals, including antimicrobials (antibiotics, fungicides, antiviral agents and parasiticides), some heavy metals, disinfectants, antiseptics, and other pharmaceuticals and natural products. AMR can occur through mutations in the genome of the microorganism or by acquiring resistant genes from other microorganisms. This latter process involves the horizontal gene transfer (HGT) of mobile genetic elements (MGEs) which carry resistant genes. These evolutionary events leading to the emergence of resistance in pathogens are rare and challenging to predict. However, they may be associated with vast ramifications when antibiotics are no longer able to treat serious infections (Larsson and Flach, 2022).

Models are being developed, which can quantify the risk of selection of AMR in the environment, including in studies from Ireland (Cahill *et al.*, 2021) and England (Stanton and Singer, 2022). These have utilised national datasets of bacterial abundance, chemical exposure and time. Datasets which can better inform these models will be expanded with increased monitoring of AMR selective chemicals and surveillance of AMR abundance.

The external environment is considered to pose a much lower risk of mutation-based evolution for AMR pathogens than within a human or animal being treated with antimicrobials (Larsson and Flach, 2022). However, Singer, Fry and Antoniou (2020) report that all resistance genes in the environment have the potential to become clinically relevant. At present, however, the threshold of AMR selective chemicals and AMR (ARB and ARG) abundance in the environment that could pose a health risk to humans or animals remains unknown (Alderton *et al.*, 2021). Larsson and Flach, 2022 also note that it remains to be determined in what environmental setting or conditions resistance genes are transferred to pathogen for the first time.

Improved understanding of the extent of the resistome is being aided by better access and affordability of sequencing technology, enabling the use of ‘metagenomics’ to study large volumes of bacterial genetic data. This technique was used in the first large-scale study of what has been termed the ‘latent resistome’, the collection or ARGs that exist but have not yet been identified to have any clinical impact (Inda-Díaz *et al.*, 2023). Significantly, the study found that the majority of these genes are already present in highly virulent human pathogens and were associated with mobile genetic elements, meaning that they are transferable to other organisms.

3.3.4 Impact: Economic Impact

An increase of resistant bacteria within environmental settings has the potential to interfere with existing agriculture and aquaculture industry practices and reduce their economic success. In turn, this would significantly impact Scotland's economy and those employed in those industries, subsequently leading to lower production of food. The economic impact of AMR is a key focus of the O'Neill report, commissioned by the UK Government (O'Neill, 2014), with a prediction of a global cost of up to US$ 100 trillion by 2050. A more recent UNEP report presented a figure of gross domestic product (GDP) shortfall of US$ 3.4 trillion annually in the next decade, which would push 24 million more people into extreme poverty (UNEP, 2023). Although Scotland-specific data on the economic impact of AMR is not available, the mortality rates and costs of treatment are likely to be approximately double for drug-resistant infections, with an estimated cost to the NHS of £180 million per year (House of Commons, 2018). However, the contributing and proportional role of the environment in such an outbreak of AMR remains unclear.

3.4 Controls for AMR in the environment

While the impact of AMR in the environment remains to be fully determined, there is an increasing focus on finding ways to control the dissemination of antibiotic resistance in the environment. These include minimising antimicrobial usage (AMU), infection prevention and control strategies, reducing the entry of ARB, ARG and AMR-driving chemicals into environment and the management of the natural environment.

3.4.1 Control: Antimicrobial Stewardship

Whilst continued use of antimicrobials is an essential component of modern medicine, the rising threat of pathogenic AMR has driven measures to optimise their use and reduce AMR risk.

Limit AMU in healthcare

In Scotland, work to reduce AMU in healthcare is coordinated by ARHAI Scotland, as a contribution to the wider UK Government 5–Year AMR NAP (UK Government, 2019). There has been a reported overall 16.9% reduction in total human antibiotic use in Scotland between 2017 and 2021 (18.8% decrease in primary care and 8.6% in acute hospital care (ARHAI Scotland, 2022). Prescription of antibiotics by pharmacists has been introduced in Scotland and England to reduce pressure on GPs. However, UKHSA note that such improved access to antimicrobials might increase AMR risk (UKHSA, 2023b).

In a Board Meeting paper published by UKHSA in September 2023, they report that in comparison to other global AMR National Action Plans, the UK ranks in the top 3 most developed country plans, with strengths in policy design, implementation, monitoring and evaluation. They also note that COVID-19 has impacted the UK's ambitions to tackle AMR, as efforts and resources were redeployed to contain the pandemic and healthcare delivery was altered (UKHSA, 2023b). In their 2022 report, the English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR) reported that the overall burden of AMR in England decreased by 4.2% between 2017 and 2021 (UKHSA, 2022).

UKHSA, formerly Public Health England (PHE), have led a number of successful UK initiatives (also known as antimicrobial stewardship efforts) to assist organisations in the appropriate AMU, including Antibiotic Guardian (UKHSA, 2023a), and TARGET toolkit for primary care and Start Smart Then Focus for secondary care. Keep Antibodies Working is a UK national public awareness campaign (UKHSA, 2023b). Similar initiatives have been introduced in Scotland, including ScRAP and Turas education resources (ARHAI Scotland, 2022). The development of online information sources offers further opportunities to deliver these initiatives.

There is growing evidence that antimicrobial stewardship (AMS) interventions to improve how antibiotics are prescribed and used are safe and effective (Davey *et al.*, 2017). However, a report published by Public Health England (2015) found key individual behaviours among healthcare professionals and the general public, which drive inappropriate AMU (e.g., lack of adherence to prescribing guidelines, unnecessary antibiotic consumption). A Cochrane Review further identified that these behaviours may be amendable to change, with recommendations that future work should focus on bringing together key stakeholders and research experts to develop more impactful AMS interventions (Davey *et al.*, 2017). One potential approach to address this urgent need is to apply behavioural science to inform the design and delivery of effective antibiotic interventions (Hulscher and Prins, 2017).

Limit AMU in agriculture

The use of antibiotics as growth promoters in animals has been banned in the EU since 2006 with a similar ban introduced in the USA In 2017 (Bloomer and McKee, 2018). The VARSS 2022 report, shows an overall reduction in the sales of antibiotics for food-producing animals in the UK between 2019 and 2022 (VMD, 2023). This translates into a reduction in sales of antibiotics by 59% since 2014. The report also notes improvement in antibiotic usage data across most livestock groups and includes veterinary antibiotic sales figures for cats and dogs, which show a 13% and 15% since 2021, respectively (VMD, 2023).

Salmon Scotland state that there is no prophylactic use of antibiotics in the salmon farming sector and that any use is supported by appropriate sensitivity testing (VMD, 2023). They further report that 95% of fish farms in Scotland are antibiotic-free. This has been made possible through the development and use of vaccines (Scottish Government, 2016). In addition, although only representative of a relatively small number of veterinary practices in Scotland, the data obtained in 2022 showed that antibiotic use in all companion animals decreased by 4.4% over the last 5 years (ARHAI, 2022).

Nobrega *et al.* (2021) study investigated food-producing animals. It focused on assessing the effects of interventions that reduced antimicrobial use in food-producing animals on the prevalence of AMR genes (ARGs) in bacteria from animals and humans. A large-scale multi-method meta-analysis was undertaken, which in summary found evidence to recommend restricting AMU in food animals.

Two reviews by the same author group Tang *et al.* (2017, 2019) also studied AMU in food-producing animals. In their first review, Tang *et al.* (2017), similar to (Nobrega *et al.*, 2021), found that interventions which restrict antibiotic use in food-producing animals are associated with a reduction in the presence of antibiotic-resistant bacteria in these animals. In a follow-up study, using their 2017 review findings, Tang and colleagues looked for evidence of “unintended consequences” of interventions that reduced the use of antibiotics in food-producing animals. They found no “consistent trends or clear harm” and recommended further evaluation is needed.

Under the Red Tractor (RT) farm assurance scheme, at least one person on each dairy farm (who is responsible for administering medicines) must undertake an approved medicines training course (RUMA, 2022a). This was a recommendation up until October 2019 when it became a full standard. In 2021, RUMA (2022a) reported that 93.2% compliance of this was achieved.

Craig *et al.* (2023) also focused on the potential for behaviour change interventions to improve antimicrobial stewardship and/or reduce inappropriate antimicrobial use (AMU). Their focus was, however, broader than (Farrell *et al.*, 2021) as they encompassed human health, animal health, and livestock agriculture stakeholders. Of note is the paucity of evidence in relation to the animal/livestock sectors – with only 11 of the 301 included studies focusing on the environmental compartment. In relation to human behaviour change, they report finding no evidence of any intervention type resulting in improvement in AMS, AMR, AMU, adherence, or clinical outcomes.

Another UK study by Hennessey *et al.* (2020) investigated in detail the usage of and resistance to antimicrobials and antiparasitics in sheep and cattle farming systems. The findings report below UK average use of antibiotics, and that agents such as tetracyclines and beta-lactams were the most commonly used. They recommend that this kind of data collection is imperative to the development of antimicrobial and antiparasitic resistance strategies in Britain.

3.4.2 Control: Improving Education & Awareness

Agriculture

A study in Scotland explored knowledge and behaviours amongst Scotland's dairy farmers around their use of antimicrobials (Borelli *et al.*, 2023). The findings demonstrate the need for more robust information sharing and education. The study identified the main barriers to responsible AMU in dairy farms were limited facilities, lack of knowledge on AMU recommendations, lack of time and financial constraints.

Guidance on animal AMU is provided through the 'Scotland Health Animals' website (NHS National Services Scotland, 2023). British Veterinary Association promote the '7 principles of responsible antimicrobial use', including avoiding inappropriate AMU, minimise use and monitoring antimicrobial sensitivity (RUMA, 2022a).

A research paper published by Pate *et al.* (2023) explored antibiotic use in 15 Scottish dairy farms. It concluded that the amount of antibiotics in use on the farms was being driven by disease prevalence. The authors noted that some farmers did not have a good awareness of antibiotics and AMR and said that they relied on their veterinarians for this advice. The study further highlights the role of improved education and antibiotic stewardship to help drive a reduction in antibiotic use.

Another review set out to assess the evidence of the behaviour of farmers and veterinarians in relation to AMU and resistance in dairy cattle (Farrell *et al.*, 2021). The researchers found that animal welfare, available resources and the relationship between farmers and veterinarians can influence decisions on the use of antimicrobials. They recommend that the use may be reduced if farmers and veterinarians collaborate more, and that interventions and research are needed on knowledge, skills, resources, and engagement.

Healthcare

In NHS Scotland, controlled use of antimicrobials in healthcare includes recommendations for shorter antibiotic courses of treatment, and adherence to the use of the AWaRe classification of antibiotics, developed by the WHO (2021b). The WHO guidance classifies antibiotics into three groups - Access, Watch and Reserve, taking into account the impact of different antibiotics and antibiotic classes on AMR. Aiming to optimise antibiotic use, prescription databases are also used to monitor trends of AMU and to provide information back to healthcare teams.

Raising public awareness of responsible AMU is also highlighted within the UK and international literature sources. For example, the Antibiotic Guardian programme, led by a website developed by Public Health England (now the UK Health Security Agency () in 2014 (UKHSA, 2023a). This initiative is delivered in collaboration with the devolved administrations of Scotland, Wales and Northern Ireland. The site encourages the public, students and educators, farmers, the veterinary and medical communities as well as professional organisations to become Antibiotic Guardians. The site includes a collection of public health educational materials, encouraging individuals to learn and understand more about AMR, and to adjust their behaviour to limit their AMU. On 8 September 2023, a total of 191,987 individuals have signed up as Antibiotic Guardians.

3.4.3 Control: Infection Prevention & Control (IPC)

Collignon *et al.* (2018) analysed antibiotic consumption and other factors driving antimicrobial resistance. They conclude that a reduction of antibiotic consumption will not be sufficient to control antimicrobial resistance because the spread of resistant strains and resistance genes appear as the dominant contributing factor. They recommend various infection prevention and control (IPC) practices for the reduction of antimicrobial resistance.

In another 2020 report, both farm-management and manure-oriented interventions reduced antibiotic use by up to 57% and reduced ARGs or antibiotic-resistant bacteria in animal waste by up to 99% (Hennessey *et al.*, 2020). This systematic review highlights that even in well-resourced agricultural settings, there are significant challenges in addressing IPC and antimicrobial resistance. Most of the evidence reviewed emerged from studies that focus on the farm itself, however, WASH and biosecurity interventions could complement each other.

The Welsh 5-year AMR Action Plan (2019-2024) (Welsh Government, 2019) includes biosecurity measures, such as safe sourcing, establishing farm boundaries, disinfecting vehicles and equipment and effective management of wildlife. The aim of these measures is to reduce the spread of disease within the agricultural setting and thus reduce the need for antimicrobials. The Scottish Government already have similar guidance for biosecurity practices for animal health (Scottish Government, 2022).

Recent work conducted by the London School of Hygiene & Tropical Medicine identifies and summarises evidence from on-farm biosecurity, water, sanitation, and hygiene (WASH) interventions with the potential to directly or indirectly reduce infections and antibiotic resistance in animal agricultural settings (Pinto Jimenez *et al.*, 2023).

Vaccines

The development of vaccines to build immunity to pathogens provides an alternative to the use of antimicrobials. In Scottish aquaculture, development of a vaccine for furunculosis in salmon in Scotland was funded / encouraged in response to the realisation that continued use of antibiotics was not a long-term viable option (UK Government, 2019). Scottish Salmon Producers Organisations worked with researchers and the UK Government. This took several years to develop but has led to the practice in the industry that today all salmon are vaccinated against this pathogen.

3.4.4 Control: Reduce Entry of Antimicrobials & AMR to the Environment

Processing of domestic and human waste through WWTPs can partially remove AMR from waste (Sambaza and Naicker, 2023). Wastewater treatment methods include Biological (activated sludge, constructed wetlands and anaerobic membrane bioreactors); Physical (filtration and adsorption) and Chemical (disinfection and oxidation) methods (Bridle, Pagaling and Avery, 2022). However, some resistant bacteria are not removed and subsequently enter the environment via discharge to surface water.

A systematic review of 1,316 individual studies by Goulas *et al.* (2020) assessed the effectiveness of various strategies used to control the dissemination of antibiotic resistance in the environment. They found that composting and drying were efficient treatments to reduce the relative abundance of ARG and MGE in organic waste, by 84% and 98%, respectively. Pei *et al.* (2019) reviewed the effectiveness of tertiary treatment technologies in WWTP for removing ARG. Chlorination, ozonation and ultraviolet treatment were not found to be ideal solutions and there are concerns that they may even increase the risk of the dissemination of antibiotic resistance in environments. In contrast, advanced oxidation process technology performed well and reduced ARGs at removal rates up to 5.8 log units. Constructed wetlands are considered a practical and cost-effective process. Other environmental management options for AMR in the environment include creating buffer zones and managing the frequency of spreading organic waste. Phytoremediation has also been suggested (Goulas *et al.*, 2020).

3.4.5 Control: Monitoring & Surveillance

Within our evidence, we identified two methods of monitoring and surveillance currently taking place in Scotland: 1) AMR (ARB and ARG) and 2) Chemicals that drive AMR selection (indirect contributors to overall AMR).

Surveillance of AMR (ARB and ARG)

Both within Scotland (Bridle, Pagaling and Avery, 2022) and internationally, there is a recognised lack of standardisation of testing methods for the surveillance of AMR in the environment. In addition, there are no standards for the permitted levels of AMR allowed to be discharged from WWTP or applied to land.

A key outcome from both the literature reviewed and expert elicitations was that there is little consistency of methods across studies and monitoring for AMR. This was also noted by Humphrey (2018) regarding methods and samples used to measure resistance in the laboratory. Both Bridle, Pagaling and Avery (2022) and Humphrey (2018) called for guidelines on standardisation or harmonisation of methods. Key conclusions from both reports were that there is a need for standard targets, standard reporting units and agreed threshold levels. The Bengtsson-Palme group advocate monitoring of ‘latent’ resistant genes as well as those currently associated with pathogens using functional metagenomics (Bengtsson-Palme *et al.*, 2023).

Bengtsson-Palme *et al.* (2023) also report the absence of comprehensive monitoring of AMR in the environment. They also highlight the lack of knowledge on ‘background’ or baseline data, which would enable monitoring to detect deviations from the normal background resistance levels in the environment. Abramova *et al.* (2023) undertook a literature survey of 150 scientific papers containing relevant qPCR data on antimicrobial resistance genes (ARGs) in environments associated with potential routes for AMR dissemination. They found that for most ARGs, the typically reported abundances in human-impacted environments are approximately one ARG copy in a thousand bacteria.

A CREW report reviewed technologies currently used for monitoring AMR in water and also identified emerging monitoring approaches, including lab-on-a-chip, sensors and miniaturised sequencing platforms (Bridle, Pagaling and Avery, 2022). Metagenomics is an emerging genetic technique that can assess resistance on a larger scale – defined as the high- throughput sequencing of microbial samples used to study bacterial communities, including the presence of resistance genes. Three of the Scottish studies have applied this method (Lepper *et al.*, 2023, and Perry *et al.*, 2019, 2021). It was noted that the most cost-effective mechanism to generate a baseline understanding of AMR in Scotland's waters was to build on existing sampling regimes and add in further sampling where specific water types weren't already covered.

In Scotland, SEPA has adopted this approach by adding measurement of AMR to their routine bathing water quality sampling. They tested resistant isolates against a range of clinically relevant antibiotics. This data show that in 2023, 0.19% of all *E. coli* samples were resistant to antibiotic cefotaxime. Their data is made accessible to the public through their website at https://informatics.sepa.org.uk/AMRmonitoring/. SEFARI report that a rapid surveillance tool is under development for the detection of pathogens resistant or susceptible to antibiotics (e.g., *E. coli*). This developing technique is predicted to shorten the turnaround time by 2-3 days for pathogen identification (Pagaling, Troldborg and Zhang, 2023).

The PATH-SAFE (Pathogen Surveillance in Agriculture, Food and Environment) programme is part of the UK's implementation of the 5–Year AMR Action Plan. One of its key aims is to improve data on the prevalence, source and pathways of food-borne disease and AMR. Examples include the characterisation of AMR *E. coli* from raw meat to identify resistance genes and circulating plasmids and an AMR wastewater surveillance pilot. Scottish partners include Food Standards Scotland (FSS) and Scotland's Rural College (SRUC). The Environment Agency in England have recently received £2 million to screen AMR in the environment over the next two years (Stanton *et al.*, 2022).

Monitoring of Drivers of AMR: Antimicrobials & Selective Chemicals

Finding ways to reduce the entry of pharmaceuticals into waterways in recognition of their detrimental impact to the environment and on development of AMR was the focus of a Scottish CREW study published in 2018 (Alejandre *et al.*, 2022). This study looked at options to introduce 'eco-prescribing' in Scotland, encourage drug choices to be made with understanding of their environmental impact. The authors highlighted that for this to be possible, there is a need for better coordination between key expert groups. They recommend a three-pronged policy framework: 1) the organisation of a coordinative mechanism between key stakeholders; 2) systematic integration of environmental criteria in formulary development supported by expert evaluation of environmental risks of pharmaceuticals; and 3) improving knowledge of healthcare workers and the public on the environment.

Within Scotland, work to reduce pharmaceutical pollution in the environment is being driven by the One Health Breakthrough Partnership (OHBP). This group brings together key regulatory, healthcare and research partners in Scotland including SEPA, Scottish Water, NHS Highland, and the Environmental Research Institute at UHI. Their focus is to find One Health innovative solutions to reduce drug use "upstream".

The Scottish review by Helwig *et al.* (2023) suggests that other 'upstream' measures to reduce the use of antimicrobials include eco-directed sustainable prescribing, the use of precision medicine to identify patient-specific medicine and dose and disease prevention. A CREW Report published in 2022 called for a need for better pharmaceutical monitoring near WWTPs in Scotland, currently only taking place near to 23% of them (Helwig *et al.*, 2022). Within this report, it is also noted that the top three water monitoring sites in Scotland are rivers and streams (58%), WWTP effluent (20%) and WWTP influent (39%). The Scottish SEFARI report recommends a greater focus on the measurement of pharmaceuticals in non-aquatic, terrestrial environments (Pagaling, Troldborg and Zhang, 2023).

Another of SEPA's visualisation tools shares information on pharmaceuticals in the water environment. This tool was developed following a CREW project, which was overseen by OHBP, to generate baseline data on pharmaceuticals in Scotland's water (Helwig *et al.*, 2022). SEPA and UKWIR's CIP (Chemical Investigation Programme) used the resulting database to generate the interactive tool[4]. This tool

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[4] **SEPA's interactive tool** can be found at:
https://informatics.sepa.org.uk/EnvironmentalPharmaceuticals/

provides the first open-access interactive tool in the UK to combine national environment and prescribing data for pharmaceuticals.

Prescription data has been used to predict the levels of pharmaceutical pollution in waters. However, there is not always a good correlation between predicted and actual levels. Researchers at Robert Gordon University in Aberdeen, Scotland, have recommended an update to an existing Data Visualisation Tool used by CIP to provide a more accurate prediction (Wagstaff and Petrie, 2023). They also provide recommendations for sample methods, for example, the collection of five random samples from a single WWTP within a calendar month.

In Scotland, a CREW study reports that in terms of pharmaceutical waste in the environment, whilst WWTW influent and effluent are well-sampled, there is little or no data on other potential sources, such as septic tanks, manufacturing effluent, landfill effluent, veterinary sources, aquaculture, and run-off from fields to which sewage sludge was applied. Most WFD water body types, other than rivers and burns, are also underrepresented (Helwig *et al.*, 2022).

The study by (Helwig *et al.*, 2022) led to the development of a database that was used to build an open-access interaction tool that combined environmental and NHS primary care prescribing data hosted on the SEPA website. The study revealed there was no data for some compounds, and that some geographical areas of Scotland were not included – for example, no data was available for 18 of Scotland's 24 Local Authority areas, with scant information on septic tanks and discharges.

Building on previous work undertaken by Teedon *et al.* (2017), which explored the experiences of communities reliant on private water supplies, the more recent report provides qualitative evidence collected in response to the work commissioned by CREW (Teedon *et al.*, 2020). This research found that extensive rural areas in Scotland are home to private water supplies and septic tanks and whilst there is obvious local expertise and individuals appear to know their own systems in most cases, significant gaps in knowledge and skill exist, particularly around how to cope with contamination, resilience planning, and fragility of water sources. The authors suggest that there is a need to undertake monitoring at a number of sites and suggest that long-term resilience planning and risk management is required for rural private supply businesses in Scotland (Teedon *et al.*, 2020).

The UK Water Industry Research's (UKWIR) Chemical Investigation Programme has included monitoring of pharmaceuticals in Scotland (CIP2), with the finding that many exceed the Predicted No Effect Concentration (PNEC) (Davison, Macadam and Smith, 2021). Antibiotics measured included macrolides, trimethoprim, sulfamethoxazole, amoxicillin and ciprofloxacin. Toxicity levels were best correlated with length of exposure, while domestic sources of pharmaceutical pollution were found to be the greater contributor, more so than trade or industry. The authors identified that eight substances, including the antibiotics clarithromycin and erythromycin, are posing the highest ecotoxicological risk in Scottish Waters (Helwig *et al.*, 2022). They suggest that Eco-directed Sustainable Prescribing (EDSP) tools should be used to guide healthcare prescribers to choose the most ecologically harmless option.

A Data Visualisation Tool has been created in Scotland by Robert Gordon University to predict the concentration of pharmaceuticals in wastewater and to determine their impact on the environment (Wagstaff and Petrie, 2023). The findings of this report demonstrate the integration of the Tool with the CIP as a means to enhance the accuracy of this prediction analysis. Recommendations to improve accuracy include a better understanding of degradation rates in sewer systems and the inclusion of data on daily wastewater flows.

Industrial Waste

Disposal of environmental pollutants represents a further external cause that can lead to the promotion of AMR in the environment. There is little data on the relative importance of this aspect for the development of AMR. This includes pharmaceuticals, personal care products, heavy metals, biocides, and plastics. They can interact with the environment through disposal into waterways or landfill sites and can promote the transfer of resistance genes. Sites of pharmaceutical production or industry are suggested to be of greater concern, where higher concentrations of potentially AMR-promoting chemicals are released in effluent (Bengtsson-Palme and Larsson, 2016). These chemicals enter waterways largely through WWTP effluents (Stanton *et al.*, 2022).

A Scottish study by CREW collated data on 60 substances in 11 different environmental matrices, with waterways being the most investigated (Helwig *et al.*, 2022). The authors report the presence of eight pharmaceuticals at concentrations of ecotoxicity. Three (clarithromycin, erythromycin, and ciprofloxacin) were identified as posing a higher risk for AMR in surface waters. One area of complexity in assessing the risk of environmental pollution on AMR is the extent of variation of the chemical cocktail present, hence challenging to determine which is responsible for selection (Stanton *et al.*, 2022).

3.4.6 Control: Policy & Research

Policy

Government-led policy has the ability to exert control on future development of AMR by setting targets and encouraging cross-sector coordination. The AMR policy in Scotland has been influenced by the UK Government's 5–Year National Action Plan (NAP) (2019 – 2024) (UK Government, 2019). This was published in follow-up to the 20-year vision. This UK NAP was developed through a collaboration of all four UK nations and follows a 'One Health' approach covering human and animal health.

The key groups who deliver on UK AMR National Action Plan actions in Scotland are ARHAI Scotland with a focus on clinical settings, providing national expertise for infection, prevention and control, AMR and healthcare associated infection, and the Scottish Antimicrobial Prescribing Group (SAPG), who work with Scottish NHS boards across health and care settings to improve antibiotic use and optimise patient outcomes. Other delivery partners include NHS Education for Scotland (NES), which is an education and training body, AMR in the Environment in Scotland Stakeholder Group (AESS), Food Standards Scotland, and SEPA with a wider role in protecting the environment and human health through environmental regulation, monitoring and reporting.

The UK AMR NAP is aligned to the key objectives of the ‘Global Action Plan on Antimicrobial Resistance’ published by the World Health Organization in 2015, which are:

- Improve awareness and understanding of antimicrobial resistance.
- Reduce the incidence of infection.
- Strengthen knowledge through surveillance and research.
- Optimise the use of antimicrobials.
- Ensure sustainable investment in countering antimicrobial resistance.

The impact of NAPs is uncertain. The global response to AMR has been described as “inadequate” (Willemsen *et al*., 2022). An assessment of action plans established to mitigate the impacts or control AMR in the environment programmes in Europe and internationally shows that although individual countries are committed to developing national action plans (NAPs), their operationalisation and delivery into impactful change at country level needs to be supported by evidence-based policy and evaluation. For example:

- Collineau *et al.* (2023) mapped and described all French surveillance programmes for antibiotic resistance. They report that the French surveillance system is fragmented, with gaps in coverage and that comprehensive mapping of the systems was difficult to do as the surveillance programmes are diverse. The authors suggest that the environmental sector, overseas territories, antibiotic-resistant-bacterial colonisation in humans and antibiotic use in companion animals are important areas that current plans cover ‘poorly’. A key recommendation is for the use of common ways across settings and countries to measure antibiotic use, resistance, and residues.

- The review by Caputo *et al.* (2023) looked at NAPs of 95 countries for content about antimicrobial resistance in aquaculture. It found that 37% of countries did not mention an aquaculture component within their AMR NAP. The South-East Asia Region had the highest implementation rate of AMR-aquaculture programmes.

- Charani *et al.* (2023) also conducted a review of AMR national action plans; however, their focus was on gaps and opportunities in strategies optimising antibiotic use in human populations. They report that the NAPs were variable and identified overall gaps as being in relation to (1) policy and strategic planning; (2) medicines management and prescribing systems; (3) technology for optimised antimicrobial prescribing; (4) context, culture, and behaviours; (5) operational delivery and monitoring; and (6) patient and public engagement and involvement.

In 2018, The Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE), and the World Health Organization (WHO) joined forces to create a Tripartite group to focus on AMR from a One Health approach. In 2022, they were joined by United Nations Environment Programme (UNEP) to form a new Quadripartite group. Since then, a number of Technical Groups have been established to address specific issues including ‘Integrated Surveillance on antimicrobial use and resistance’ and ‘Economics of Antimicrobial Resistance’. Table 4 summaries the timeframe of key international, UK and Scottish policy related to AMR.

Table 4. Timeline of key AMR policies and strategies 2015 – 2023

Date	International	UK	Scotland
2023	A One Health priority research agenda for antimicrobial resistance published by the Quadripartite Group		Scottish Healthcare Associated Infection (HCAI) Strategy 2023 – 2025 published by Scottish Government
2022	One Health Joint Plan of Action 2022 – 2026 published by Quadripartite Group UNEP joins existing Tripartite group (WHO, FAO and WOAH) to form the Quadripartite partnership	Tackling antimicrobial resistance 2019 to 2024: addendum to the UK's 5-year national action plan published by UK Government	
2019		Tackling antimicrobial resistance 2019–2024: The UK's five-year national action plan published by UK Government The UK's 20-year vision for antimicrobial resistance published by UK Government	
2018	MoU between Tripartite Group members to cooperate to combat health risks at the human–animal–environment interface		
2017	A European One Health Action Plan against Antimicrobial Resistance (AMR) published by the European Commission Frontiers 2017: Emerging Issues of Environmental Concern published by UNEP		OHBP group formed 'Scotland's Healthy Animals' website launched by Scottish Government
2015	Global Action Plan on Antimicrobial Resistance published by WHO		

2014		Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations published by O'Neill (Review commissioned by UK Government)	Scottish Management of Antimicrobial resistance Action Plan 2014 – 18 (ScotMARAP 2) published by Scottish Government

UK's Exit from the European Union

Following the UK's withdrawal from the European Union in January 2020, the UK Government released an addendum in 2022, setting out amendments to their 2019 AMR National Action Plan. Out of the 17 new commitments added, of particular relevance are proposed revisions to:

- Improve the surveillance of AMR and antimicrobial use.
- Improve the availability of data to better understand the prevalence of AMR across human health and animals.
- Explore and evaluate AMU, prescribing, new therapeutics, diagnostics, stewardship and resistance across both human health and animals.
- Promote the UK's lead role in AMR interest and political grouping internationally.

AMR relevant changes within the EU that have taken place since, include the EU Commission's adoption of a proposal to strengthen EU action against AMR to "combat AMR in the fields of human, animal and environmental health" in June 2023. The recommendations focus on:

- Infection prevention and control.
- Surveillance and monitoring.
- Innovation and availability of efficient antimicrobials.
- Prudent use and cooperation among Member States and globally.

Of particular note is the key change to AMU in agriculture made by the EU in January 2022.

Regulation (EU) 2019/6 on veterinary medicines and Regulation (EU) 2019/4 on medicated feed introduced a ban on the routine use of antibiotics in farm animals and on the import of meat, dairy, fish and eggs produced using antibiotics to stimulate rapid growth. With the UK no longer subject to this regulation, it means that the following are still legal practice in the UK but not in the EU:

- Giving antibiotics to farm animals routinely, rather than when they are sick or have an infection.
- Giving preventative group treatments to farm animals.
- Giving antibiotics to farm animals to compensate for inadequate welfare standards, lack of care or poor hygiene.
- Importing animal foods produced with antibiotic growth promoters.

Despite the lack of introduction of any similar legislation in the UK, there has been continued reduction of antibiotic use in agriculture in line with the UK NAP. The potential impact of imported meats from other countries that may occur from new non-EU trade deals, however, remains to be determined. In the UK, Responsible Use of Medicines in Agriculture (RUMA), is a multi-partner agriculture alliance with members across the UK, whose aim is to promote the responsible use of antibiotics in agriculture and aquaculture (RUMA, 2023). Their 26 members include Scottish representation from the National Farmers Union Scotland and Salmon Scotland.

AMR Research

The Scottish One Health AMR register (SOHAR) provides a comprehensive overview of research activity in Scotland related to AMR (Holden *et al*., 2021). It shows that the main efforts are relatively evenly distributed among the main topics of Animal Health, including Epidemiology and AMR usage; Clinical AMR; and Detection and Surveillance, whilst Transmission from Food or from Wildlife are least represented. Key recommendations for future research in AMR that emerged from this work are:

1. To maintain and reinforce the strengths of AMR research activities in Scotland.

2. To address less well-represented areas related to food, environment and translational applications of product development.
   a) Continue to invest in international quality science, related laboratory capacity for AMR surveillance, and environmental contamination.
   b) More work is required to understand the contribution of food in the development of clinically relevant resistance, and the potential flow of AMR through the whole food supply chain.
   c) Increase representation from the environmental science disciplines, including for water, and investigate the role of agricultural soils and the link to agronomic practices in the potential transmission of resistant microbes and associated pharmaceuticals and other environmental pollutants.
   d) The translation of the fundamental science to end-user applications, either for alternatives to antimicrobials for treatments or diagnostic tools needs further consideration. This could be achieved through establishing stronger partnerships with commercial and NHS partners.

Three key multi-agency research groups in Scotland, whose work contributes to furthering knowledge on AMR are CREW (Centre of Research for Waters) and OHBP (One Health Breakthrough Partnership) and SEFARI (Scottish Environment, Food and Agriculture Research Institutions).

CREW is a Scottish Government funded partnership between the James Hutton Institute, Scottish Higher Education Institutes and Research Institutes, established to provide expert advice on policy in relation to water quality and health and provide evidence needed to influence and implement future policy.

OHBP is a collection of researchers and experts across five organisations within healthcare, environment and water sectors: NHS Highland, SEPA, Environmental Research Institute, Scottish Water and CREW. They are focused on the reduction of pharmaceutical pollution in the environment through sustainable One Health innovation. Their mission is to reduce the environmental impact of healthcare practises, influence policy and to drive research and innovation. They align closely with the Scottish Government's 'Hydro Nation Agenda'. 'Pharmaceuticals in the Environment' and 'Antimicrobial Resistance' are two of the OHBP workstreams.

SEFARI's work includes the recent development of an 'AMR systems map' for Scottish research. The map illustrates that most of the known transmission pathways are being researched, with a focus on resistance in farmed animals, and in environmental habitats. The key research findings reported to date are:

- A new mathematical modelling method developed helps determine how sampling can be carried out efficiently and identify likely predictors of AMR in livestock.
- The development of a Scottish nationwide map of resistance gene abundances in rural soils shows that while some forms of resistance are an endemic part of microbial ecology in soils, others are localised. The work identified that genetic diversity is driven by environmental factors (e.g., heavy metals) and agricultural practices.
- A rapid surveillance tool is under development for detection of pathogens resistant or susceptible to antibiotics (e.g., *E. coli*). This developing technique is predicted to shorten turnaround time by 2-3 days for pathogen identification.
- SEFARI researchers are tracking the presence of AMR genes in the food chain The ongoing work has uncovered multiple mechanisms of action for the bacteria *Camplyobacter jejuni*.
- Survey work is now underway to determine whether seals carry AMR genes and to characterise antibiotic-resistant *Campylobacter*.

We found two reviews which set out evidence on the use of research to assess and monitor AMR in the environment. Spets, Ebert and Dinnétz, 2023 have looked at the potential for spatial analysis of AMR and (Painter *et al.*, 2023) undertook a review to quantify and highlight what they see as an important evidence gap. They found limited evidence of interventions addressing AMR in the environment having been subjected to economic evaluation.

Work by Khan in 2018, preceded these reviews of AMR NAPs, but also focused on AMR strategic programmes and activity. Their systematic review analysed 100 One

Health Networks (OHNs) in Africa and Asia, and identified limited representation of stakeholder engagement, potential for duplication of effort across networks, and absent or unclear monitoring and evaluation approaches. Five years ago, they found that 32 OHNs covered only human and animal health, without engaging with the role of the environment on health (Khan, 2018).

Projected Future Controls

SEFARI in Scotland highlight a need for greater focus on the measurement of pharmaceuticals in non-aquatic, terrestrial environments (Pagaling, Troldborg and Zhang, 2023).

Wastewater-based epidemiology (WBE) offers a promising technique to help monitor AMR. WBE involves the measurement of chemicals in wastewater effluent to provide information on levels of chemicals or microbials in waterways as a means of providing community-wide epidemiological data. It has been suggested this technique could be used to monitor antimicrobial drugs, resistance genes and cofactors in a population-wide basis in wastewater (Sims, Avery and Kasprzyk-Hordern, 2021). This would provide a new layer of surveillance, beyond the current focus on clinical and prescription data.

CREW undertook a review of literature on WBE (Sims, Avery and Kasprzyk-Hordern, 2021). They found that other current uses of WBE other than infectious disease tracking include monitoring illicit drug use, estimation of disease prevalence based on pharmaceutical drug detection, markers of lifestyle (by measuring chemicals such as alcohol and nicotine). The use of WBE to monitor SARS-COV-2 represented a combined effort between Scotland, England and Wales, water companies and researchers. The infrastructure established from the COVID-19 pandemic presents an opportunity to use WBE for monitoring AMR.

Public Health Scotland are developing a 5-year Pathogen Genomics One Health Strategy for Scotland through the Public Health Microbiology Strategy for Scotland. However, there are currently no regulations on antibiotic concentrations in the environment. Regulatory limits were proposed by Bengtsson-Palme and Larsson (2016), who suggested that "emission limits for antibiotics must be set individually for each compound, and that different antibiotics have very different potential to be selective". Workstream 4 of the PATH-SAFE programme, which includes FSS as a Scottish partner, has been established to develop an IT platform for environmental AMR, to coordinate data across the UK collected from humans and animals and build a One Health surveillance system for AMR.

In addition to anthelmintics, a method for the determination of anticoccidials in the aquatic environment, including those present at very low concentrations has been developed and used for investigating groundwaters across Ireland. Findings reported the first detection of anticoccidials in groundwaters in Ireland, which are commonly used in poultry production, with monensin as a feed additive and amprolium as a medicine (Mooney *et al.,* 2020). Poultry activity (i.e., farming or manure spreading) was reported as a major driver behind anticoccidial presence in groundwaters.

4. CONCLUSIONS

This review has identified the key causes, impacts and controls for AMR in the environment that are relevant to Scotland. The main causes or sources that provide entry for AMR into the environment are human and agricultural waste. Regarding the impact, the environment functions as a pathway for transmission of AMR to humans, animals and crops and as a reservoir where AMR evolution can occur. This latter is enhanced by the presence of AMR-selective chemicals in the environment. The review indicates that the most frequently used controls to respond to AMR threat, including its presence in the environment, include optimised and responsible AMU in healthcare and agriculture, control of the entry of AMR into the environment from these sources, and surveillance of AMR and Antimicrobial selective chemicals in the environment. Using the review questions as a framework, we set out what can be concluded from the current literature on AMR in the environment and highlight identified gaps relevant to Scotland. We also outline areas of horizon scanning of note.

Our literature search resulted in 59 sources of evidence with direct relevance to Scotland. We did not identify any high-quality Scottish-specific evidence (e.g., systematic reviews, meta-analyses or randomised controlled trials). The literature, in terms of its topic focus and quality was very diverse, including only 3 reviews (2 narrative and 1 mini-review), 21 grey literature reports, and 19 original research publications, with the remaining a collection of thesis, webpages, conference reports, pamphlets and information notes. The different study types associated with the 59 included publications can be seen in Appendix Three.

The Scottish research gathered and presented here is not necessarily of good quality but does summarise the current research landscape on AMR in the environment. Also, as a result of the limited number of studies, the evidence is not distributed evenly across environmental compartments and causes/impacts/controls but is generally clustered. This may have created bias in the conclusions drawn.

More specifically, it is evident that water environments were the most researched AMR reservoirs (24), followed by livestock (13), healthcare (10), and food (7). In contrast, although some research focused on more than one reservoir, studies investigating soil, wildlife, sludge, sewage, manure and companion animals were underrepresented. We did not identify any publications on air. This area remains significantly under-researched in Scotland.

Overall, the focus of each literature source and the environmental compartments investigated were extremely varied – estuarine, coastal, surface freshwater, effluent wastewater, wastewater treatment plants, agricultural soils and animals, and the ‘environment’ or ‘natural environment’ overall.

Of the reports, 11 were authored by multi-sector stakeholder groups that have developed a leading role in driving forward research of AMR in the environment in Scotland including CREW (Centers of Expertise for Waters), SEFARI and OHBP. The remainder were from individual agencies that also play key regulatory roles in Scotland, including SEPA, ESS, SRUC and ARHAI Scotland.

As illustrated by the Heatmap of Scottish Evidence (see Appendix Three), all environmental AMR Scottish references focused on 'Controls' of AMR (59), and 42 discussed research. The largest subcategory within 'Controls' was surveillance of either AMR or AMU (26) and there were also a good number of studies on monitoring of AMR selective chemicals (17). Fewer studies addressed control at the cause/source of AMR entry into the environment (6) and there was only one, which addressed IPC as a means of controlling AMR. Twenty-nine studies had engaged with the 'Impact' of AMR (29), with an equal number addressing each of the sub-category – environment health (8), transmission (10) and evolution (8). The majority of the studies that addressed the 'Causes' of entry of AMR into the environment focused on human waste (9), with only 3 looking at farm waste (agriculture/aquaculture).

4.1 Causes of AMR in the Environment

The rise of infectious pathogens that are resistant to treatment by antimicrobials is considered a significant global health threat. The environment is considered important in AMR for 2 reasons 1) as a reservoir of ARB and ARG, and 2) by providing pathways (e.g., water, soil or air) through which these ARB and ARG can spread. In this section, we discuss the 'causes' (also often referred to as sources) that can contribute to the presence of ARB and ARG in the environment and consider their nature scope, scale and frequency.

4.1.1 Known Causes

Cause: Human Waste

Human waste is considered a significant cause, or source, of entry of AMR genes and AMR carrying bacteria into the environment. A Scottish report highlights that domestic and hospital wastewater contains excreted ARB, ARG and an estimated 30-100% of unmetabolised antimicrobials (Pagaling, Troldborg and Zhang, 2023). This has led to a research focus on human waste as a **significant source of AMR**

to the environment, particularly **WWTPs. Four Scottish studies** investigated human wastewater, including three primary research (Lepper *et al.*, 2023 and Perry *et al.*, 2019, 2021) and one report (Hough *et al.*, 2021). Findings show that treated WWTP effluent retains some antimicrobials, ARB and ARG from waste and so can help drive AMR selection in the environment. **Two other Scottish studies**, including a PhD thesis and primary research, support those findings by reporting that hospitals act as a reservoir and 'enrichers' of resistance (Knight, 2022; Day *et al.*, 2019).

Combined sewer overflows (CSO) are used at times of sewage network overload with rainwater and lead to the disposal of untreated sewage into waterways. There are 3,614 CSOs within the 50,000km of the sewer network in **Scotland** operated by Scottish Water and regulated by SEPA (Scottish Water, 2023a). In addition to CSO outfalls listed on Scottish Water network licenses, the Forth Rivers Trust have mapped 122 outfalls of unknown sources in the River Almond catchment area, of which 19 showed **signs of pollution**. Our review found that the impact of CSOs in AMR selection is understudied but that this is an area of increasing public, policy and scientific interest.

The use of WWTP sewage sludge (also known as biosolids) as a fertiliser for agricultural land is another potential route for AMR entry. SEPA, 2019 report that over 70% of sludge produced in Scotland is used in this way. One Scottish report found that **sewage sludge samples reviewed contained a high concentration of ARBs** (Hough *et al.*, 2021). Based on the evidence gathered, the dangers of AMR within sludge remain unclear and require **further investigation**. Another potential source of AMR from human waste is septic tanks associated with private water networks.

We did not find **any Scottish-specific evidence investigating CSOs**, **biosolids or septic tanks** within our evidence pool. Furthermore, as highlighted by one report, little data exists on monitoring septic tanks (Helwig *et al.*, 2022). Given the potential of untreated sewage to exert significant AMR pressure in the environment, this is an area that should be more closely investigated for an AMR role.

The overall findings of the studies reviewed indicate that **surface water environments have the potential to act as AMR reservoirs**. The evidence also highlights the potential role that streams, and bathing waters may have in AMR dissemination throughout the aquatic environment. The potential for **AMR contamination of the aquatic environment** by faecal waste from gulls, dogs and other wild animals was also highlighted, as well as the need for further exploration into the contribution and impact such wastes may have on the environment. The presence of clinically significant ARB and/or ARGs in surface waters, many of which may carry resistance to multiple antimicrobials, may ultimately pose a serious risk to human health.

Cause: Agriculture / Aquaculture

Livestock waste contains ARB and ARGs. The direct application to agricultural land and subsequent run-off into surface water is also considered a significant introductory route for AMR (UKHSA, 2022). Agriculture is a key industry for Scotland with approximately 80% of Scotland's land used for agriculture. A Scottish conference poster reported **low AMR levels in healthy Scottish sheep** sampled from abattoirs and field flocks (Tongue *et al.*, 2018). This is **in contrast to a more recent empirical study** of Scottish cattle herds, where a high herd-level prevalence was estimated for a proxy of AMR, with regional differences, including the highest prevalence in the Northeast and Southeast of Scotland and the lowest in the Highlands (Fernández Rivas *et al.*, 2021). As most dairy production takes place in lowland grassland areas in the Southwest of Scotland, this area requires further attention.

The condition of Scottish seas, coasts and estuaries has been reported to be in good or excellent condition, with significant reductions in pollution over the last 25 years (Scotland's Environment, 2014). The aquaculture industry is significant, with Scotland being the third biggest producer of farmed Atlantic salmon in the world. Salmon Scotland, the leading industry body, reported that **95% of farms in Scotland did not use any antibiotics in 2021**. However, antibiotic usage in Salmon aquaculture has increased by 168% between 2017 and 2021 and was the second highest among UK food producing animal species (RUMA, 2022a). This is important as the aquaculture industry (majority from salmon farming) produce represents 40% of Scotland's food production (Salmon Scotland, 2023).

4.1.2 Gaps & Horizon Scanning - Causes

The review of the literature identified four main issues projected to further impact the known causes of AMR. These are as follows.

Climate Change

The changing weather patterns in Scotland are projected to bring about hotter, drier summers; and milder, wetter autumns and winters (NatureScot, 2023). Although there are some benefits to that (e.g., longer growing season), there are concerns about resulting damaging effects, including reduced field access for grazing, manure spreading and cultivation, reduced crop and grass yields, more crop disease and damage from insects, new and more aggressive pests and diseases in crops and animals (e.g., liver fluke in cattle and sheep), increased soil erosion and shrinking habitats (NatureScot, 2023).

Higher temperatures, increased rainfall and sea level rise put pressure on the environment and, in turn, on society (NatureScot, 2023). As a result, the risk of flood increases, leading to increased use of CSOs, water demand may start to exceed supply in places, and wildlife can suffer as rivers heat up and water levels fall. Drier summers bringing drought could potentially lead to **increased chemical and bacterial concentration in waterways** and cause waterborne disease outbreaks (WHO, 2022). This is also an international concern with the ability of warmer climates to increase the transmission of disease from animals to humans (zoonoses) and to change the existing natural habitats of vector-borne diseases, as noted in a recent WHO report (2022).

Global travel

The increasing movement of humans around the globe **raises the potential for the spread of emerging pathogens**, with the air providing a key environmental transmission route for respiratory pathogens (WHO, 2022). The fast international spread of a new pathogen for which there is no available treatment was demonstrated during the COVID-19 pandemic. With international travel, individuals are at risk of infection and colonisation with multi-drug-resistant organisms and therefore can contribute to the further spread of AMR. A recent review of evidence has shown that approximately 30% of international travellers acquire antibiotic-resistant bacteria during travel, predominantly *E. coli*, and often carry resistance to multiple antimicrobials (Sridhar *et al.*, 2021).

A study of toilet waste from 18 international aeroplanes arriving in Copenhagen, Denmark, from nine cities in three world regions have been found to contain ARG. Waste from planes was found to carry resistance to critically important antibiotics from South Asia to North America (Petersen *et al.*, 2015). Efforts are required to address the increase in AMR associated with global travel and prioritise routine genomic surveillance. International travel of companion animals carrying AMR could present another potential source of AMR. AMR has also been found in the ballast water of ships. As the discharge of ballast water from ships is currently the main pathway leading to the invasion of alien organisms through offshore waters, this is also a possible source of AMR in Scottish coastal waters (Guo *et al.,* 2022).

Population size and demographics

Changes to the size and movement of populations have the potential to impact the sources of AMR. For example, increased population and seasonal variation of numbers in both urban and rural areas can add pressure to existing sewage networks. This is of **particular relevance to Scotland** with its busy summer seasons of visitors. Globally, there is a risk that environmental degradation and the increased density of the human population, global trade and international transportation will intensify the emergence of new zoonotic diseases (WHO, 2022).

In dense populations, zoonotic pathogens are more likely to be transmitted by direct contact with living or dead animals, also by feeding on animals and their products, or indirectly from the environment, via soil, water and air. A key contributing factor to these pathways is environmental degradation (WHO, 2022).

Major Global Events

Since the beginning of the 21st century, there has been a range of emerging global zoonotic outbreaks, including severe acute respiratory syndrome (SARS) coronavirus (2003), Ebola virus (2005 and again in 2017), swine flu (2009), influenza H1N1 (2009), Zika fever (2015), Middle East respiratory syndrome coronavirus (2015) and COVID-19 (2019). The recent events, such as the COVID-19 pandemic and the war in Ukraine have been highlighted as potential impacts on AMR (UK Risk Register, 2020). For example, the **COVID-19 pandemic led to increased use of antibiotics** for hospitalised patients. While these drugs were not effective against the virus itself, their use was essential to protect the patients from secondary infection such as pneumonia. Early data from **hospitals in England, Scotland and Wales** showed that 85% of COVID-19 patients received one or more antibiotic during their admission (Russell *et al.*, 2021).

Data on infections following war wounds during the current conflict in Ukraine is not yet publicly available. However, cases of antimicrobial-resistant microorganisms reported in people with war wounds were first described in military conflicts in Iraq and Afghanistan around 15 years ago, and further reported in Ukraine during the 2014-2020 period (Abou *et al.,* 2023). **Antibiotic prescribing for dental patients in Scotland rose by 49%** following the suspension of routine dental care in 2020. The data also show that since the remobilisation of NHS dental care, antibiotic prescribing remains raised at levels around 28% higher than pre-pandemic (Duncan *et al.*, 2021).

Antifungal resistance (AFR)

There is also an identified need for further investigation of resistance to antimicrobials other than antibiotics, in particular **resistance to antifungals** which, as highlighted by England's Environment Agency in 2022, is an emerging area of concern (Environment Agency, 2022c). A UK and Ireland-wide study, including **three sites in Scotland**, found *Aspergillus fumigatus* isolates resistant to an azole antifungal antimicrobial. Using phylogenomic analysis they identified antifungal-resistant (AFR) isolates of near-identical genotypes from both environmental and clinical sources, indicating with high confidence the infection of patients with resistant isolates transmitted from the environment (Rhodes *et al.*, 2022).

4.2 Impact of AMR in the Environment

The environment functions as a reservoir, where AMR accumulates and is disseminated across a landscape. The environment also functions as the substrate for ecological processes, which drive the evolution of organisms. Here, we conclude what is known from the literature about the scope, scale and frequency of these impacts.

Impact: Health of the Environment

It is not clear how, or whether, AMR (AMR genes or AMR-carrying microorganisms) could impact the health of the environment. AMR presents a risk to the health of people, animals and crops by making antimicrobials ineffective. A resistant organism in the environment becomes a problem if it causes infection in humans, animals or crops. However, if the infection is not treated (e.g., in wild animals and plants), it has no direct consequence, whether the infectious agent is resistant to antimicrobials or not. When assessing AMR impact on the health of the environment, it is important to always **distinguish the direct ecological effects of antimicrobial pollution with the effects of AMR** (AMR genes or AMR-carrying microorganisms). There is some evidence that antimicrobials and AMR selective chemicals in the environment may cause adverse effects directly on the health of the environment.

Impact: Transmission of AMR to Humans, Animals and Crops

The ability of the **environment to act as a pathway for the transmission** of resistant pathogens to humans, animals and crops, causing illness or death, is a leading impact of concern. These events are understood to occur commonly, and although challenging, they may be quantifiable and relatively predictable. Because only one individual will be infected in each transmission event, the consequences are limited. A recent systematic review of international evidence found 40 studies that provided empirical evidence of health outcomes in humans associated with exposure to antibiotic resistance in the natural environments (Stanton *et al.*, 2022).

Contaminated waterways are a commonly researched pathway of pathogen transmission from the environment. For example, WWTP and CSO effluent have the potential to contaminate recreational and bathing water with resistant microbes. SEPA's annual surveillance has detected **AMR *E. coli* at 58-78% of Scotland's designated bathing waters.** Drinking water contamination is another key concern. One Scottish study detected **resistance to sulphonamides** and markers for transferable genetic elements in tap water in Glasgow. About 3% of the Scottish population uses private water supplies (PWS) for drinking water, mostly in remote and rural areas. These PWS are reported to have a higher level of faecal indicator failures than public supplies and in this way, represent an increased risk of AMR transmission to humans.

The presence of AMR pathogens in **food** (meat and crops) represents another potential route of transmission to humans. **AMR has been detected in raw Scottish retail meat samples**. These pathogens are likely to represent a low risk due to heat treatment during cooking. Despite concern about contaminated fresh produce such as fruit and vegetables, which are not always heat-treated, there were **no AMR *E. coli* detected** in 80 samples of Scottish fruit and vegetables from a variety of retail outlets. In addition, evidence exists on **association between wildlife and transmission of bacterial pathogens and/or AMR to the food chain**. This highlights the need for continued monitoring and assessment of this risk in Scotland, particularly around migratory bird populations (Greig *et al.*, 2015).

Air is another potential environmental medium of AMR transmission, particularly for respiratory pathogens. Antifungal resistant (AFR) isolates with near-identical genotypes were isolated from both environmental and clinical sources, indicating with high confidence the infection of patients with resistant isolates transmitted from the environment. As demonstrated with COVID-19, airborne pathogens can transmit very quickly and cover vast geographic areas. In their extensive review, (Stanton and Singer, 2022) report that AMR in air environments is under-researched. However, there are **no studies to date investigating this environmental compartment in Scotland**.

Impact: Evolution of AMR Within the Environment

Bacteria (and other microorganisms) in the environment can acquire resistance following contact with non-pathogenic environmental bacteria that carry resistance genes (natural resistome) or through contact with resistance bacteria that have been introduced through human or animal waste. **Resistance develops due to selective pressure from a variety of AMR** selective chemicals which are released into the environment through human activity. The evolution of AMR giving rise to the emergence of novel resistance, or to increased fitness of resistance pathogens. These evolutionary events are regarded as rare and challenging to predict. However, they may be associated with vast ramifications when antibiotics are no longer able to treat serious infections. Models are being developed to quantify the risk of selection of AMR in the environment. These have utilised national datasets of bacterial abundance, chemical exposure and time.

Impact: Economic Impact

Persistence of pathogenic AMR within environmental compartments that could lead to infection in humans and animals has the potential for significant economic impact on healthcare, agriculture and aquaculture. A 2017 World Bank report estimated a gross domestic product (GDP) shortfall of US$ 3.4 trillion annually in the next decade as a result of AMR, which would push 24 million more people into extreme poverty. Although Scotland-specific data on the economic impact of AMR is not available, the mortality rates and costs of treatment are likely to be approximately double for drug-resistant infections, with an estimated cost to the NHS of £180 million per year (House of Commons, 2018). However, the **contributing and proportional role of the environment in such an outbreak of AMR remains unclear**.

4.3 Control of AMR in the Environment

The emerging threat of resistant, untreatable pathogens has driven the introduction of controls to optimise the use of antimicrobials in healthcare and agriculture, the two leading areas of antimicrobial use. More recent focus on environmental AMR has led to the consideration of further controls to limit the entry of AMR-driving chemicals, AMR bacteria and AMR genes into the environment. The ability to measure the efficacy of these controls is complicated by the rich cocktail of AMR drivers found in the environment; the lack of current understanding of concentrations of these that select for AMR; and the lack of standardisation in monitoring and surveillance methods. These challenges are driving future research to address these knowledge gaps. The description of controls to mitigate the risk of AMR in Scotland is provided in Table 5.

Table 5. Key controls of AMR in Scotland

Type of Control	Description of Control	Current status in Scotland
Responsible AMU	Reduce and optimise AMU in healthcare, agriculture and aquaculture to remove this selective pressure on development of ARB and ARG. Driven by policy.	Scotland has driven down healthcare AMU in recent years. Public and professional awareness of responsible AMU has improved. Mutli-stakeholer approach with leaders including NHS and RUMA.
Alternatives to AMU	New drugs, use of vaccines, infection prevent & control	IP&C in healthcare, biosecurity in agriculture / aquaculture. Vaccines.
Monitoring & Surveillance	Monitoring & surveillance of the levels of AMR and AMU. Waterways are of primary focus as WWTP effluent from human waste provides highest concentration of ARB, ARG and AMR-selective chemicals to surface waters. Work ongoing to	CREW & OHBP are multi-stakeholder groups leading studies on waterways and working with a One Health approach. UK-wide studies with Scottish input include UKWIR CIP & Environment Agency (England) studies.

	standardise methods and to use this to introduce controls.	
Policy & Research	Improve awareness and understanding of AMR; reduce the incidence of infection; improving the availability of data on the prevalence of AMR; explore and evaluate new therapeutics and diagnostics; and encourage cross-sector coordination. The main research efforts are Animal Health, Clinical AMR; and Detection and Surveillance.	Key groups who deliver on UK AMR NAP actions in Scotland are ARHAI Scotland and CARS, with partners SEPA and Food Standards Scotland. SOHAR Register provides a comprehensive overview of AMR research activity in Scotland.

4.3.1 Current Controls

Controls: Limit AMU in Healthcare

Continued use of antimicrobials is an essential component of modern medicine. However, the rising threat of pathogenic AMR has driven measures to optimise their use and reduce AMR risk. In the UK, these measures are currently led by the national 2019-2024 5-year AMR Action Plan, created in collaboration with all devolved nations. Scotland's implementation of this plan is coordinated by ARHAI Scotland through the Scotland One Health National Antimicrobial Resistance Action Plan (SOHNAAP) group. Delivery partners include Scottish Antimicrobial Prescribing Group (SAPG), NHS Education for Scotland, SEPA, AMR in the Environment in Scotland Stakeholder Group (AESS) and Food Standards Scotland. ARHAI work includes publication of an annual report (SONAAR) on AMR and AMU and resistance in humans, animals and the environment (ARHAI Scotland, 2022). The success of these measures is demonstrated by an overall **16.9% reduction in total human antibiotic use in Scotland between 2017 and 2021**, with decreases of 18.8% in primary care and 8.6% in acute hospital care (ARHAI Scotland, 2022). Prescription databases are used to monitor trends of AMU and to provide information back to healthcare teams.

However, **further ongoing action is needed to mitigate the threat of AMR**. The recent UKHSA (2022) report reveals that the estimated total number of serious antibiotic-resistant infections in England rose by 2.2% in 2021 compared to 2020 (53,985 compared to 52,842) (UKHSA, 2022). Scottish data found that AMR levels in clinical infections caused by *E. coli* and *Klebsiella pneumoniae* have remained stable between 2020 and 2021, however, a high level of vancomycin resistant *E. faecium* have been reported (40%) in blood isolates (ARHAI Scotland, 2022). Specific targets have been introduced to optimise the AMU, reduce specific drug-resistant infections, and expand the use of diagnostic tests (UKHSA, 2022). An important lesson that emerged from the COVID-19 pandemic is **the importance of rapid, accurate and affordable diagnostics** to manage the outbreak and develop appropriate, evidence-based policy responses. The same applies to AMR. One key area that remains underexplored is the use of **behavioural and social science** in the design and evaluation of interventions to improve antimicrobial use in healthcare settings (Hulscher and Prins, 2017).

Controls: Limit AMU in Agriculture & Aquaculture

International scientific evidence indicates that AMU in animals may contribute to AMR in humans (Tang *et al*., 2017). Although AMU treatment of livestock and farmed fish is essential to maintain welfare, reduction of use will help limit AMR development and therefore, the potential dissemination to the environment from these industries. Data on AMU in animals has historically been based on sales data published annually by VARSS. In the UK, RUMA is a multi-partner agriculture alliance with members across the UK (including representation from NFU Scotland and Salmon Scotland), that promote the responsible use of antibiotics in agriculture and aquaculture. The recent VARSS 2022 report shows that UK antibiotic sales for food-producing animals have reduced by 59% since 2014 (VMD, 2023)

The use of antibiotics as growth promoters in animals has been banned in the EU since 2006. In the UK, under the Veterinary Medicines Regulations 2013, **antimicrobials are a prescription-only medicine** prescribed by a veterinary surgeon after a clinical assessment of the animal. The recent data from Veterinary

Medicines Directorate (2023) show an **overall reduction in antibiotic use in freshwater and marine farms**. However, although usage in salmon decreased in 2022, it remains 15% above 2017 usage. There was also an increase in antibiotic use in trout farms between 2021 and 2022, which VMD (2023) attributed to an outbreak of a disease on a small number of production sites. In addition, ARHAI Scotland (2022) report that overall antibiotic use in companion animals is also reducing over time in Scotland.

Data on Scotland's dairy farms shows that the amount of antibiotics in use on the farms is driven by disease prevalence (Pate *et al*., 2023). However, there is **a need for improved education, information sharing and antibiotic stewardship among farmers** to help reduction in antibiotic use (Borelli *et al*., 2023). To support farmers and veterinary surgeons involved in farm health planning, Scotland's Rural College (SRUC) Veterinary Services, funded by the Scottish Government, developed web-based software Scottish Animal Health Planning System (SAHPS) and an associated data capture phone app. The app, which free to Scottish veterinary practices and farmers, links to SAHPS, allows farm data to be shared in real time.

Information and guidance on AMU in animals for farmers, vets and animal keepers is provided by the 'Scotland's Healthy Animals' website (NHS National Services Scotland, 2023). British Veterinary Association promote the '7 principles of responsible antimicrobial use'. Another key control measure to reduce reliance on AMU in animals is through **biosecurity**. As detailed within the Welsh 5-year AMR Action Plan, this includes safe sourcing, establishing farm boundaries, disinfecting vehicles and equipment and effective management of wildlife. The aim of these measures is to reduce the spread of disease within the agricultural setting and so reduce the need for antimicrobials.

Controls: Monitoring & Surveillance

Our review identified two methods of monitoring and surveillance currently taking place in Scotland: 1) AMR (ARB and ARG), and 2) chemicals that drive AMR selection, which are indirect contributors to AMR.

A key finding is the **need for standardisation of comprehensive testing methods for the surveillance and monitoring of AMR** in the environment in Scotland (Bridle, Pagaling and Avery, 2022) and internationally. We identified that there is a need for standard targets, reporting units and agreed threshold levels. Bengtsson-Palme *et al. (*2023), who are a leading international group on AMR, advocate monitoring of 'latent' resistant genes as well as those currently associated with pathogens using functional metagenomics.

Work is ongoing in Scotland to improve the current monitoring of AMR in water and the use of metagenomics to assess resistance on a larger scale is showing promising results. Three Scottish studies that used this method highlighted the need to build on existing sampling regimes and add in further sampling where specific water types were not examined (Lepper *et al.*, 2023; Perry *et al.,* 2019 and 2021). Of note are the 'lab-on-a-chip' technologies, which have been designed to be portable, user friendly and more easily used in field settings (Bridle, Pagaling and Avery, 2022). A **rapid surveillance tool**, which is currently under the development for detection of pathogens resistant or susceptible to antibiotics (e.g., *E. coli*), is predicted to shorten the turnaround time for pathogen identification from 3 days to as quick as a few hours (Pagaling, Troldborg and Zhang, 2023).

Across the UK, other surveillance and monitoring activities include the PATH-SAFE programme, which aims to improve data on the prevalence, source and pathways of food-borne disease and AMR. Examples include the characterisation of AMR *E. coli* from raw meat to identify resistance genes and an AMR wastewater surveillance pilot. Scottish partners include Food Standards Scotland (FSS) and Scotland's Rural College (SRUC).

4.3.2 Gaps & Horizon Scanning - Controls

Increased surveillance of WWTPs

A proposed EU update to the Urban Wastewater Treatment Directive (UWWTD) requires that from 2025, monitoring of AMR is in place for WWTP and beginning in 2035 Quaternary treatment is introduced to urban wastewater in order to eliminate the broadest possible spectrum of micro-pollutants (European Commission, 2022). It is estimated that up to 80% of removal, calculated on the basis of set of pollutants, should be achieved (European Commission, 2022; Pistocchi *et al.,* 2022). Wider application of **WBE** which has been used for monitoring public health and lifestyle and for COVID-19 could be used to monitor AMR.

Standardisation of testing methods

UK Government's Risk Register (2020) recognises **the need to strengthen lab capacity and surveillance systems**, to enable fast identification and tracking of AMR threats and to provide tools to interpret and share this information. This will involve innovation and collaboration in academic and commercial sectors. There is a need to monitor other water compartments including lochs, estuaries, surface water and groundwater with these bodies of water commonly used for recreational, drinking, irrigation and agricultural purposes. Increased monitoring of soil and air is also needed. Ireland has reported the need to incorporate anticoccidial surveillance in groundwater quality monitoring (Cahil *et al*., 2021; Monahan *et al*., 2021. UKHSA recognise the **need to enhance whole genome sequencing (WGS)**[5] **for detecting AMR genes in bacterial samples** and strengthen their bioinformatic capacity for analysis to interpret data. Inequalities in testing coverage and laboratory quality assurance programmes within and between countries are likely to contribute to AMR variance in data from country to country.

Improved Pharmaceutical Monitoring

The 2022 CREW report highlights that, in Scotland, there is currently **no pharmaceutical monitoring in 18 of Scotland's 32 Local Authority areas**. This includes the three Island authority areas where there may be a potential impact of chemicals used within the aquaculture sector. Also, of 453 NHS sites in Scotland, pharmaceutical monitoring has only taken place near 40% of these (Helwig *et al.,* 2022).

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[5] **WGS** – whole genome sequencing is a comprehensive method for obtaining detailed information about bacteria

Improved diagnostics

There is a recognised **need to improve diagnostic tools** that can more quickly and accurately identify pathogens to better inform and target antimicrobial use. This will include improvements in the capacity of the UK's reference labs to test bacterial responses to antimicrobials. Innovation in microbial testing methods and equipment is expected to bring improvements, such as the use of loop-mediated isothermal amplification (LAMP) and Lab-on-a-Chip tools for rapid testing and detection of disease outbreaks that can deliver faster turnaround times and be used in point-of-care settings (Bridle, Pagaling and Avery, 2022).

Development of new drugs

Development of new drugs is an international requirement and one that will impact all nations. Within England, the antimicrobial purchasing project piloted by NHS England and the National Institute for Health and Care Excellence (NICE) is a subscription-style payment model for antibiotics which pays pharmaceutical companies upfront for access to their product based on its value to the NHS, as opposed to the volume used (UK Government, 2020). This is predicted to **help incentivise the development of new classes of drugs across the UK** for the first time in 30 years. Development of new drugs is particularly important where resistance to 'last resort' antibiotics has already developed, for example for some strains of tuberculosis (UK Government, 2020). Over the past decade, over 50 similar international and national initiatives aimed at incentivising antibiotic research and development have been implemented, including the Joint Programming Initiative on Antimicrobial Resistance, New Drugs for Bad Bugs and Biomedical Advanced Research and Development Authority.

Research

The environment by its scale impacts a range of research and regulatory areas. For this reason, it is essential the stakeholders with expertise work together to scale the immense challenge of furthering our understanding of environmental AMR and its associated health and economic risks. **CREW and OHBP are two examples of successful partnership working**, bringing together key Scottish stakeholders to improve knowledge and inform policy. CREW's Scottish One Health AMR register (SOHAR) provides a comprehensive overview of research activity in Scotland related to AMR (Holden *et al.,* 2021). It shows that the main efforts are relatively evenly distributed among the main topics of Animal Health, including Epidemiology and AMR usage; Clinical AMR; and Detection and Surveillance, whilst Transmission from Food or Wildlife are least represented. PATH-SAFE and CIP are examples of UK-wide research programmes helping to investigate AMR and inform future policy and action direction.

An example of a **European partnership initiative is ANTIVERSA**, a study across seven European nations exploring whether biodiversity can act as an ecological barrier to limit the spread of AMR. In 2023, the leading international policy Quadripartite group (which includes WHO, UNEP, FAO and WOAH) published their 10 highest-priority research areas which are focused within the five pillar areas of transmission, integrated surveillance, interventions, behavioural insights and change, and economics and policy (FAO *et al.*, 2023). The **UK also offers a strong research base**. For example, UKHSA led a study published in 2019 reporting on the global burden of AMR using data from 204 countries (Murray *et al.*, 2022).

European Partnership on One Health Antimicrobial Resistance (EUP OH AMR), a co-funded research and innovation (R&I) partnership, is expected to start in 2025 under the Horizon Europe R&I framework programme (Joint Programming Initiative on Antimicrobial Resistance, 2023). It will deploy a joint research programme, co-funded by the EUP OH AMR partners and the European Commission (EC). In Scotland, animal health and welfare, except for the veterinary profession, veterinary medicines and international negotiations, is devolved to **Scottish Ministers, who work closely with a range of stakeholders**, other UK administrations, and regulatory and enforcement authorities to develop evidence-based policies (Scottish Government, 2023).

Policy

A One Health AMR response that considers animals, humans and the environment is now at the forefront of international policy. Within the UK, this is currently driven by the 20-year vision and the 5-year AMR action plan (2019-2024). This covers human and veterinary medicine, food production, agriculture and research. Wales introduced a 5-year plan (2019-2024) for AMR in animals and the environment, aligning with the UK plan. One of their five aims is to focus on the need to better understand the role of the environment in the development and spread of AMR and to monitor antibiotics in the environment, particularly water sources. Within the UK, **all nations including Scotland are involved in the development and implementation of wider UK policy on AMR**.

The next round of UK policy on AMR (NAP for 2024-2029) will be the first to be developed outwith European membership. It sets out (as part of a One Health approach) a set of commitments for the surveillance of AMR and AMR-driving chemicals in air, land and water environments, and the use of this evidence to inform interventions to minimise the spread of AMR in the environment (SEPA, 2023d). This is something that requires close monitoring and, as a partner UK nation, **Scotland has the opportunity to contribute to these discussions**. In addition, it would be expected that the UK would continue involvement in global surveillance initiatives, such as the Global Antimicrobial Resistance and Use Surveillance System (GLASS), given that international data-sharing and partnership working are essential to fight the global risk of AMR (WHO, 2023).

As demonstrated by the COVID-19 pandemic, **infection control measures can vary widely** from country to country. In November 2022, the UK Government began a call for evidence on AMR to inform the next 5-year NAP: 2024 – 2029. A total of 195 responses were considered from private, public and non-profit sectors, 40 of which were from Scotland and 30 from outside the UK, with healthcare representing the predominant sector. 81% of respondents felt that the threat of AMR had increased since 2019. The 2024 to 2029 national action plan will be published in

2024, subject to ministerial agreement across the UK government and in Northern Ireland, Scotland and Wales.

On 1 June 2023, the European Parliament adopted a resolution on EU action to combat antimicrobial resistance. Since the UK is no longer a member state of the EU, one **area of key attention is the direction of future UK policy regarding the control of AMU in agriculture**. Previous examples of EU-led regulation include the 2006 ban on the use of antibiotics as growth promoters in animal feeds. More recently, in January 2022, the EU banned the import of meat, dairy, fish and eggs that have been produced using antibiotics to stimulate rapid growth in the animals.

In summary, the role of the environment in the development and spread of AMR is globally recognised as complex and not wholly understood (Larsson *et al.*, 2023). It is evident from the results of this review that further research is needed to better understand the role of the environment in AMR evolution and transmission, in Scotland and for the scale and nature of potential impact on human health and the health of the environment itself to be better quantified and monitored.

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APPENDIX ONE: Full search strategy applied in MEDLINE

Key: TX = All Text   MH = Exact Subject Heading

Medline (EBSCOhost) Search conducted 7 Sept 2023

#	Query	Results
1	(MH "Anti-Bacterial Agents")	402,725
2	(MH "Anti-Infective Agents")	63,842
3	TX "Anti biotic" OR antibiotic OR "antibiotic resist*" OR antibacterial	645,318
4	(MH "Drug Resistance, Bacterial") OR (MH "Drug Resistance, Microbial") OR (MH "Drug Resistance, Multiple, Bacterial")	133,525
5	TX " anti microbial" OR antimicrobial OR "antimicrobial resist*" OR AMR OR "antibiotic resistant gene*" OR ARG OR resistome OR mobilome	372,659
6	S1 OR S2 OR S3	770,229
7	S4 OR S5	454,728
8	(MH "One Health") OR (MH "Environment") OR (MH "Environmental Monitoring") OR (MH "Environmental Microbiology") OR (MH "Environmental Pollution") OR pollutant OR (MH "Water") OR (MH "Wastewater") or (MH "Sewage") OR slurry OR sludge OR effluent OR (MH "Rivers") OR coast* OR (MH "Estuaries") OR (MH "Aquaculture") OR animal OR (MH "Cattle") OR cow or (MH "Sheep") OR (MH "Swine") OR pig OR (MH "Poultry") OR (MH "Livestock") OR (MH "Animals, Wild") OR wildlife OR (MH "Zoonoses") OR (MH "Food") OR (MH "Food Chain") OR (MH "Soil") OR (MH "Soil Microbiology") OR (MH "Composting") OR compost OR (MH "Manure") OR (MH "Air")	8,525,684

	OR (MH "Aerosols") OR bioaerosol OR (MH "Farms") OR (MH "Agriculture") OR (MH "Crops, Agricultural") OR (MH "Plants")	
9	monitor* OR comply OR compliance OR evaluat* OR surveillance OR detect* OR regulat* OR policy OR governance OR framework OR "risk assess*" OR standard* OR quality OR control* OR stewardship OR prevent* OR impact* OR implement*	17,279,872
10	source OR pathway OR cause OR causation OR system	8,017,303
11	(MH "Meta-Analysis as Topic") OR TX (meta analy* or metaanaly) OR TI ("systematic review"OR review)	1,098,170
12	S6 OR S7	991,753
13	S8 AND S12	250,233
14	S8 AND S9 AND S12	151,346
15	S8 AND S10 AND S12	77,824
16	S13 OR S14 OR S15	250,233
17	S11 AND S16	5,793
18	S7 AND S8 AND S11	3,095
19	S7 AND S8 AND S9 AND S11	2,115
20	S7 AND S8 AND S10 AND S11	1,342
21	S19 or S20	2,526
22	S19 or S20 Limiters - Date of Publication: 20160101-20230831	1,952

APPENDIX TWO: Key AMR projects currently ongoing in Scotland

Project / Collaborating Group	Funding Organisation / Partners
CREW: Centre of Expertise for Waters	Scottish Government funded partnership between James Hutton Institute, Scottish Higher Education Institutes and Research Institutes, Scottish Water, SEPA, NatureScot, Drinking Water Quality Regulator, Scottish Canals, Food Standards Scotland, Zero Waste Scotland, Water Industry Commission for Scotland, Consumer Scotland
Glasgow Caledonian University: Protecting and improving surface and ground water resources.	Co-funded by the Scottish Government in partnership with CREW
Moredun Research Institute: The transmission pathways of pathogens between livestock, wildlife, people and the environment	Charity registered
RESAS 22-27: A Systems Understanding Of The Flow Of AMR From Livestock Production To The Environment And Humans: Informing Risk Analyses	Scottish Government: Rural & Environment Science & Analytical Services
RESAS 22-27: A systems understanding of the flow of AMR from livestock production to the environment and humans: information antimicrobials stewardship and optimal use	Scottish Government: Rural & Environment Science & Analytical Services
ROADMAP to foster transitions towards prudent use of antimicrobials in animal	Scottish Government funded in partnership with James Hutton Institute.

production in different contexts to manage antimicrobial resistance	
SEFARI: Flows of antimicrobial resistance and pathogens through environment to food chain	Scottish Government funded in partnership with James Hutton Institute.
SEFARI: Scottish Environment, Food and Agriculture Research Institutions. A consortium of one Centre for Knowledge Exchange and Innovation Gateway	Scottish Government funded in partnership with Biomathematics and Statistics Scotland, The James Hutton Institute, Moredun Research Institute, The Rowett Institute, Royal Botanic Garden Edinburgh, Scotland's Rural College
SEFARI: Understanding the dynamics antimicrobial resistance genes flux in the soil, animals and humans in different fertilisation practices in grasslands	Scottish Government
OHBP: One Health Breakthrough Partnership	Scottish Government's Water Industry Division funded in partnership with NHS Highland, SEPA, Environment Research Institute at UHI, Scottish Water, CREW
PATH-SAFE: Tracking Foodborne Pathogens and Antimicrobial Resistance Microbes	HM Treasury's Shared Outcomes Fund
University of Edinburgh: The study of the dynamics and spread of AMR at the veterinary-human interface (One Health)	Co-funded by the Natural Environment Research Council (NERC), UK-China Newton fund, and Joint Programming Initiative on Antimicrobial Resistance (JPIAMR; Horizon2020)

APPENDIX THREE: Heatmap of Scottish Evidence

The Heatmap has been ordered chronologically, starting with the most recent publication and then alphabetically for each year.

Lead author, year **Type of publication, title**	**Environmental Compartment**	**AMR area addressed**													
		CAUSE			**IMPACT**				**CONTROL**						
		General	Agriculture / Aquaculture waste	Human waste (WWTP)	General	Health of environment	Transmission pathways	Evolution of AMR	General	IPC	At Cause/source	At use	AMR/AMU Surveillance	AMR Selective Chemicals	AMR Research
Borelli, E. 2023 Primary research study: Antimicrobial usage and resistance in Scottish dairy herds: a survey of farmer's knowledge, behaviours and attitudes.	Livestock											√			√
Helwig, K. 2023 Review: Broadening the Perspective on Reducing Pharmaceutical Residues in the Environment.	Water, Healthcare			√		√					√	√		√	√
Lepper, H. 2023 Primary research study: Distinctive hospital and community resistomes in Scottish urban wastewater: Metagenomics of a paired wastewater sampling design.	Water			√									√		√
National Services Scotland, 2023 Webpage: Scotland's Healthy Animals	Livestock, Companion animals									√		√			
One Health Breakthrough Partnership, 2023 Webpage: OHBP Homepage	Water											√		√	√
Pagaling, E. 2023 Report: The transport, fate and impact of pharmaceuticals in the environment in Scotland.	Water			√		√		√						√	√
Pate, L. 2023 Primary research study: Factors influencing Scottish dairy farmers' antibiotic use.	Livestock											√			√
Scottish Environment Protection Agency, 2023 Report: Antimicrobial resistance (AMR) in the environment SEPA Information Note.	Water	√			√				√						

Scottish Government, 2023 Government document: Scottish Healthcare Associated Infection (HCAI) Strategy 2023- 2025.	Healthcare								✓						
Scottish Government Veterinary Services Programme, 2023 Report: Antimicrobial resistance in healthy livestock.	Livestock												✓		
Vallejo-Trujillo, A. 2023 Understanding source attribution, infection threat and level of AMR of *E. coli* in Scotland using whole genome sequencing.	N/A												✓		✓
Wagstaff, T. 2023 Report: Estimation of pharmaceutical concentrations in wastewater using the data visualisation tool factors affecting measured and predicted influent pharmaceutical wastewater concentrations.	Water													✓	✓
Alejandre, J. 2022 Report: Environmentally informed pharmaceutical prescribing in Scotland: current policy landscape and proposed policy options to enable the implementation of eco-directed pharmaceutical prescribing in the Scottish healthcare system.	Water, Healthcare					✓						✓		✓	✓
Antimicrobial Resistance and Healthcare Associated Infection Scotland (ARHAI). 2022 Scottish One Health Antimicrobial Use and Antimicrobial Resistance (SONAAR) report (2021).	N/A												✓		
Bestwick, C. 2022 Pamphlet: SEFARI: Leading ideas for Antimicrobial Resistance.	All								✓						✓
Bishop, H. 2022 Primary research study: Bacteriological Survey of Fresh Minced Beef on Sale at Retail Outlets in Scotland in 2019: Three Foodborne Pathogens, Hygiene Process Indicators, and Phenotypic Antimicrobial Resistance.	Food						✓						✓		✓
Bridle, H. 2022 Report: Technologies for Monitoring and Treatment of Antimicrobial Resistance in Water.	Water										✓		✓		✓
Elsby, D. 2022 Primary research study: Antimicrobial resistant Escherichia coli in Scottish wild deer: Prevalence and risk factors.	Wildlife, Food												✓		✓
Environmental Standards Scotland, 2022 Report: Baseline Evidence Review - Population, human health and cultural heritage (Strategy and Analysis).	Healthcare	✓			✓				✓						
Food Standards Agency, 2022 Report: Pathogen Surveillance in Agriculture, Food and Environment Programme (PATH-SAFE).	Food						✓						✓		
Helwig, K. 2022 Report: Pharmaceuticals in the water environment: baseline assessment and recommendations.	Water							✓						✓	✓
Knight, M. 2022 Thesis (PhD): Anthropogenic and natural factors influencing the prevalence and persistence of antimicrobial resistance genes in the Scottish environment.	Wastewater, Soil, Manure												✓		✓

Metcalf, R. 2022 Primary research study: Sewage-associated plastic waste washed up on beaches can act as a reservoir for faecal bacteria, potential human pathogens, and genes for antimicrobial resistance.	Sewage			√			√						√		√
Niemi, L. 2022 Primary research study: Spatiotemporal trends and annual fluxes of pharmaceuticals in a Scottish.	Water													√	√
Scottish Environment Protection Agency, 2022 Press Release: New data tool launched to help researchers understand the effects human medicines have on Scotland’s environment.	Water													√	
Davidson, H. 2021 Report: Pharmaceuticals in freshwater environments and their potential effects on freshwater invertebrates.	Water					√								√	√
Djuwanto, B. 2021 Thesis (MRes): Wild birds as a reservoir of antimicrobial resistance (AMR) in the environment.	Wild birds												√		√
Elsby, D. 2021 Thesis (PhD): Using wildlife to biomonitor for antimicrobial resistance in the Scottish environment.	Wildlife Food												√		√
Fernández Rivas,C. 2021 Primary research study: High Prevalence and Factors Associated With the Distribution of the Integron intI1 and intI2 Genes in Scottish Cattle Herds.	Livestock		√				√						√		√
Holden, N. 2021 Report: Scottish One Health AMR register (SOHAR): one health research into antimicrobial resistance (AMR): a register of the research literature, projects and collaborations in Scotland.	Water, Livestock, Food, Healthcare														√
Hough, R. 2021 Report: Community Concerns regarding the Impacts on Human Health and the Environment arising from the spreading of Sewage Sludge to land.	Sludge					√		√			√				
Hough, R. 2021 Report: Identifying environmental priorities and analytical requisites for Environmental Standards Scotland.	Water	√			√				√						
Hough, R. 2021 Report: Human Health Risk Assessment of Potentially Hazardous Agents in Land-Applied Sewage Sludge.	Sludge			√			√	√			√				√
Humphry, R. 2021 Primary research study: Estimating antimicrobial usage based on sales to beef and dairy farms from UK veterinary practices.	Livestock											√			√
Perry, M. 2021 Primary research study: Secrets of the hospital underbelly: patterns of abundance of ARGs in hospital wastewater vary by specific antimicrobial and bacteril family.	Wastewater, Healthcare			√									√		√
SEFARI Gateway, 2021	Livestock, Food								√						√

Webpage: Antimicrobial resistance: bringing Scottish expertise together to find the solutions.															
Sims, N. 2021 Report: Review of wastewater monitoring: applications for public health and novel aspects of environmental quality.	Wastewater												✓	✓	✓
Pollock, J. 2020 Review: Alternatives to antibiotics in a One Health context and the role genomics can play in reducing antimicrobial use.	Livestock											✓			✓
Niemi, L. 2020 Primary research study: Assessing hospital impact on pharmaceutical levels in a rural 'source-to-sink' water system.	Water			✓		✓					✓			✓	✓
Niemi, L. 2020 Blog: Pharmaceuticals in the environment: Introduction and cross-sector partnership addressing the issue in Scotland.	Water					✓								✓	
Teedon, P. 2020 Report: Private water supplies and the local economic impacts in Scotland.	Water								✓		✓				
Day, M.2019 Primary research study: Extended-spectrum β-lactamase-producing Escherichia coli in human-derived and food chain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study.	Food						✓						✓		✓
Knapp, C. 2019 Report & Dataset: Antibiotic resistance genes found in soils across the entire Scottish landscape 2007-10.	Soil												✓		✓
Perry, M. 2019 Primary research study: Antimicrobial resistance in hospital wastewater in Scotland: a cross-sectional metagenomics study.	Water			✓									✓		✓
Rogers, K. 2019 Primary research study: Can the legacy of industrial pollution influence antimicrobial resistance in estuarine sediments?	Water							✓						✓	✓
Humphry, R. 2018 Report: Measurement of antimicrobial usage & resistance.	Livestock											✓	✓		✓
Tongue, S. 2018 Conference poster: Bla Bla black sheep, have you any AMR?	Livestock		✓				✓						✓		✓
Khan, S. 2017 Primary research study: The use of minimum selectable concentrations (MSCs) for determining the selection of antimicrobial resistant bacteria.	N/A							✓						✓	✓
Helwig, K. 2016 Primary research study: Ranking prescribed pharmaceuticals in terms of environmental risk: Inclusion of hospital data and the importance of regular review.	Water					✓		✓						✓	✓
Khan, S. 2016 Primary research study: Antibiotic Resistant Bacteria Found in Municipal Drinking Water.	Water						✓						✓		✓
Khan, S. 2016	Water						✓						✓		✓

Primary research study: Relationship between antibiotic- and disinfectant-resistance profiles in bacteria harvested from tap water.															
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