Antimicrobial resistance surveillance strategies within wild flora and fauna of England

Chief Scientist's Group report

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Dr Robert Bradburne
Chief Scientist

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Executive summary

Antimicrobial resistance (AMR) is one of the most serious global health threats facing society, with bacterial AMR alone predicted to be responsible for around 1.27 million deaths in 2019. The problem of AMR is widespread, occurring in clinical, veterinary, agricultural, and environmental settings. To tackle this, a holistic “One Health” approach is required to address the threat of AMR. Wild animals and plants host diverse communities of microorganisms, including species that are pathogenic to both humans and domesticated plants and animals. In some cases, wild animals and plants represent potential routes of transmission of AMR to human and domestic animal populations. As a result of their potential role in AMR transmission and their ability to act as integrators of environmental exposure, wildlife has the potential to be used as sentinels for AMR in the environment.

This review aimed to investigate the potential for wildlife surveillance to contribute to our understanding of the One Health dimensions of AMR. This was achieved by evaluating a selection of existing wildlife surveillance programmes in England, as well as reviewing the current literature on AMR in wildlife (including wild animals and plants) to identify key AMR hosts and markers for surveillance.

We identified and collected information on 13 wildlife surveillance schemes, covering both wild animal and plant targets, that either currently, or have the potential to, collect information of AMR. There were significant biases in the taxonomic targets of existing surveillance schemes, with animals being better represented than plants. Whilst a few wildlife surveillance schemes were ranked highly in terms of their existing surveillance structure and potential to perform AMR surveillance, there was a lack of schemes that are currently collecting information on AMR in wildlife. Only two schemes that deployed active surveillance collected microbial data (the Environment Agency’s Fish Disease surveillance and Forest Research’s plant health surveillance), and there were no active surveillance schemes that collected data on AMR.

A semi-systematic survey was performed into published literature on AMR and wildlife. A final database of 453 publications was interrogated to identify the geographic study location, the taxonomy of the target host wildlife species, the environment type, the analytical methods used, the rationale for the study and whether the host species was present in the UK. Our review identified significant geographic, taxonomic, and method-based biases in the literature. Specifically, among all wildlife surveyed, mammals and birds far outweighed other taxonomic groups, with only five of 453 publications investigating AMR in wild plants. The review highlighted priorities for future research to better understand how and where AMR exists and is transmitted in wild populations of animals and plants.

Finally, we present a series of knowledge gaps and recommendations for possible wildlife-AMR surveillance schemes to better understand the potential for AMR to act as sentinels of AMR in the natural and anthropogenic environment.

1 Introduction

1.1 Background

Antimicrobial resistance (AMR) is one of the most serious global health threats facing society, with bacterial AMR alone predicted to be responsible for around 1.27 million deaths in 2019 (Murray et al., 2022). The problem of AMR is widespread, occurring in clinical, veterinary, agricultural, and environmental settings. As a result, it has been proposed that a holistic “One Health” approach is required to address it (Velazquez-Meza et al., 2022), which aims to balance and optimise the health of people, animals, and ecosystems, rather than just focus on clinical settings.

AMR naturally exists in the environment, both in environmental microbial communities (“microbiomes”) and in animal and plant associated communities. However, AMR above natural background levels, in the form of increased abundances of resistant microorganisms and resistance conferring genes, can occur in the environment in response to anthropogenic activity. This includes the release of resistant microbes from anthropogenic compartments such as wastewater and agriculture, as well as the release or application of antimicrobial resistance-driving chemicals, which can include antimicrobials (antibiotics, antifungals, antivirals, etc.), metals, biocides, and other environmental pollutants (Stanton et al., 2022a). Not only is the presence of AMR in the environment of importance in a One Health context, but it has also been shown that clinically relevant AMR can emerge from an environmental origin (Poirel et al., 2002). There is concern that elevated levels of AMR and resistance driving chemicals in the environment may result in reduced human health outcomes, however there is only a small (yet growing) body of evidence explicitly demonstrating this (Stanton et al., 2022b).

Although the environmental dimension of AMR has historically been overlooked, it has become a rapidly growing area of research for the last decade. A small component of this research has focused on the role of wildlife, including animals, and to a lesser extent plants, as mechanisms for the dissemination, emergence, and persistence of AMR. Wild animals and plants harbour interconnected microbiomes, within which diverse communities of microorganisms exist, including species that are pathogenic to both humans and domestic plants and animals. The intermittent or chronic exposure of wildlife to sources of pollution and resistant organisms can result in temporary or permanent changes to these microbiomes, including carrying elevated levels of resistant microorganisms and antimicrobial resistance genes (ARGs). As such, wildlife is a potential reservoir of disease-causing microorganisms and ARGs, as well as a hotspot for horizontal gene transfer (HGT) (Eckert et al. 2016). Wildlife also represents a transmission pathway between anthropogenic and environmental compartments, either when hunted or foraged as sources of food, or by facilitating the movement of resistant microorganisms between hosts. For these reasons, investigating the extent and drivers of AMR in wildlife is necessary to explore how human activity modifies AMR within wildlife populations, and to better understand the role that wildlife play in the wider One Health context.

The ability of wildlife microbiomes to reflect their surroundings means they can act as "environmental integrators", incorporating the effects of exposure to environmental contamination, including AMR and resistance driving chemicals. The use of wildlife as indicators of ecosystem health is widely established and used by environmental regulators to inform surveillance of ecosystem health; for example, targeting fish, aquatic plant, diatom, or invertebrate communities. The use of wildlife specifically as sentinels for AMR in the environment is less common, but has the same potential advantages, in that organisms may better reflect average environmental exposures. Using wildlife may reduce some of the complexity involved with directly sampling environmental matrices, such as water, that are prone to significant biological and chemical fluctuations over short spatial and temporal extents (Rode et al., 2016). Some research efforts have suggested that sentinel organisms (e.g., bivalve molluscs (Grevskott et al., 2017) and small mammals (Furness et al., 2017)) could be used for AMR surveillance in the environment. However, given that our understanding of the dynamics of AMR in wildlife is still in its infancy, it is largely unknown what types of organisms and what ecological and trophic characteristics are important when choosing sentinel targets.

There are a wide range of analytical methods available for monitoring AMR in environmental samples, with many focusing on quantifying the levels of resistance at the pollution source (e.g., wastewater treatment works and farms) and in receiving environments. These monitoring methods can include culturing of indicator bacteria and/or fungi, and antibiotic resistant bacteria (ARB), quantifying resistance genes by quantitative polymerase chain reaction (qPCR), and analysis of microbial communities and their genes by 16S rRNA (bacteria) and Internal transcribed spacer (ITS)(fungi) gene sequencing, metagenomic and metatranscriptomic approaches (Anjum et al., 2017). For longer-term monitoring efforts, it is critical to identify the best methods to address the aims of the surveillance effort, as a lack of consistency and focus on monitoring efforts can lead to inconsistencies in research outputs and thus policy interventions.

1.2 Report aims

To assess the potential for wildlife surveillance to contribute to our understanding of the One Health dimensions of AMR, this report aims to evaluate a selection of existing wildlife surveillance efforts in England as a starting point for such a programme. The report will also review the current literature on AMR in wildlife (including wild animals and plants) to identify key AMR hosts and markers for surveillance. Specifically, the report aims to:

1. Review existing wildlife surveillance activities, or other potential data sources and other mechanisms which exist that do, or could, collect information on the presence of resistant microorganisms in wild animals and plants of England.
2. Review the literature relating to AMR in wildlife to identify how different species and methods are used in AMR surveillance. This will include an overview of reservoirs and/or transmission routes between important receptors (crops, humans, livestock etc.) and the environment.
3. Identify any knowledge gaps relating to aims 1 and 2.

4. Make recommendations for new sample method collections from selected species that would enhance our knowledge of AMR in the environment.

2 AMR monitoring in wild flora and fauna

2.1 AMR surveillance in wild flora and fauna

Surveillance for microbial pathogens is defined as “the ongoing systematic collection, collation, analysis and interpretation of data and the dissemination of information to those who need to know in order for action to be taken” (World Health Organization, 2001). The well-established epidemic (e.g., Ebola) and pandemic (e.g., SARS-CoV-2) risks associated with disease transmission from animals to humans means that the need for wildlife surveillance of zoonotic diseases is steadily increasing. However, AMR in wildlife (in both pathogenic and non-pathogenic organisms) is less frequently the focus of surveillance activities and research. There are considerable knowledge gaps concerning the dynamics of resistant microorganisms within wildlife populations, including and understanding of the natural levels of AMR in wildlife, how human activity influences AMR, and how AMR is transmitted between wildlife and other reservoirs.

Surveillance for AMR could be implemented as an extension of existing wildlife pathogen surveillance activities, “to identify changes in the infection and/or health status of animal and human populations” (Halliday *et al*., 2007). However, as well as being present in pathogenic microorganisms, AMR is widely present in commensal and non-pathogenic microorganisms. Given the drivers of AMR in wildlife can come from chemical sources, as well as horizontal gene transfer (HGT) from non-pathogenic microorganisms, surveillance efforts for AMR would benefit from a broader focus than one simply focused on the specific genes and their microbial host. AMR wildlife surveillance could holistically include (adapted from McCluskey *et al*., 2003; Halliday *et al*., 2007):

1. Understanding transmission risks of AMR microorganisms from wild flora and fauna to human and domestic flora and fauna.
2. Understanding the impact of anthropogenic activity on AMR into wild flora and fauna.
3. Detection of changes in the prevalence or incidence of AMR carrying microorganisms or genes over time.
4. Testing specific hypotheses about the ecology of AMR in wild populations.
5. Evaluating the efficacy of potential AMR control interventions.

The objectives for surveillance will play an important role in the design and structure of a surveillance scheme, as it is unlikely that a single scheme or target species can answer all questions simultaneously.

2.2 Wildlife surveillance types

Wildlife surveillance primarily takes the form of passive or active surveillance (Halliday et al., 2007; Neo & Tan, 2017). Passive surveillance often involves unstructured collection of samples or reporting of data from a variety of sources, including other regulatory authorities and citizen scientists, based on a set of reporting criteria. For example, wildlife pathogen

surveillance frequently relies on submission of disease data from the animal and plant sector to veterinary authorities. Such data include illnesses or deaths in animals and plants, or notifiable infectious diseases that must be reported by law. These data are then used to determine disease trends, identify potential outbreaks, or monitor for new disease threats. Active surveillance involves surveillance of wild populations of plants or animals in a more structured manner, both spatially and temporally. For wildlife disease, active surveillance provides more detailed information on disease prevalence, as both healthy and unhealthy individuals are often targeted for analysis. An alternative form of active surveillance, described by Neo & Tan (2017) is the use of sentinel organisms, described as "a naïve animal which is intentionally placed in an environment of potential infection that is monitored at short time intervals to detect infection".

The choice of active or passive surveillance will depend on the exact objectives of the monitoring programme, and the financial and time resources available. Passive surveillance tends to be lower cost than active surveillance, as samples and/or individuals for analysis are supplied in an *ad hoc* manner. The focus on diseased, dying, or dead individuals in passive surveillance programmes means that there will be bias towards diseased individuals. In an AMR context, this may mean that passive surveillance is less suitable for understanding overall levels of AMR prevalence in populations, where AMR is harboured by commensal organisms, but could be used to assess emerging risks and pathogen spillover. Passive surveillance is frequently the least time-consuming, labour-intensive, and expensive of the surveillance methods.

Well-designed active surveillance schemes have the potential to provide a wider range of information due to the structured nature of data collection, including information on AMR prevalence within populations, changes in AMR in relation to specific land-use characteristics or events (such as disease outbreaks), and long-term change over time. Moreover, by not being constrained to diseased organisms or collected carcasses, active surveillance can generate a less biased understanding of AMR in populations, which can help to assess both acute and long-term health. However, active surveillance tends to be more time- and resource-intensive, and requires a long-term commitment from funders, meaning that this type of surveillance is less commonly deployed. In addition, there are also practical issues, for example, some wild flora and fauna species may not be practical to sample due to their ecology, rarity, or behaviour, meaning that passive surveillance is the only practical approach, despite its potential drawbacks.

2.3 Evaluating wildlife surveillance schemes

To understand whether wildlife surveillance schemes in England currently, or have the potential to, collect data on AMR in wildlife, we undertook an evaluation of a range of existing wildlife surveillance schemes. To structure the assessment and comparison of schemes, standardised scheme evaluation criteria were used.

The availability of several kinds of data within existing wildlife/flora surveillance efforts can be helpful for understanding the characteristics of the scheme and its appropriateness to be used for AMR surveillance, these include:

1. The type of organism (e.g., mammal, bird, fish, reptile, invertebrate, plant etc.).
2. The trophic level (e.g., predator, herbivore, omnivore, etc.).
3. The habitat (e.g., terrestrial, freshwater, marine, etc.).
4. The purpose of the survey (e.g., population or disease surveillance).
5. Sample type collected (e.g., individuals, faeces, etc.).
6. Type of surveillance (e.g., active or passive).
7. How long the scheme has been running for.

Our aim was to develop an evaluation scheme to identify existing monitoring programmes that either perform AMR surveillance or have characteristics that make them suitable for future AMR surveillance. Due to the wide range of potential survey aims, the evaluation scheme below was not designed to address all AMR surveillance aims, but to provide guidance on the broad criteria to be considered when identifying existing wildlife targets or schemes that are suitable for the surveillance of AMR. The following subheadings describe the main characteristics of surveillance schemes that are relevant of AMR monitoring in wildlife, and what features could be prioritised. These are also summarised in Table 1 below.

2.3.1. Species distribution and abundance

An important characteristic for wild flora or fauna surveillance is both the abundance and geographic distribution of the target species. A species, or group of species, that is abundant enough that surveillance can be conducted in a cost-effective manner is preferable to one that is less common and harder to find and collect data from. Spending a large amount of time and effort in surveillance of a rare target is unlikely to be an effective use of resources. Likewise, species that have a widespread distribution are generally preferable to species with localised distributions. Focusing on species that have limited distribution, or specialised habitat preferences, would limit the ability to generate representative data at a national scale. As a result, we have defined four categories to represent this characteristic:

1. National and abundant.
2. National and low abundance.
3. Regional and abundant.
4. Regional and low abundance.

2.3.2. Frequency of sampling

The frequency at which samples are collected is an important factor. Irregular or low frequency (e.g., annual) sampling will be less informative than higher resolution (seasonal, monthly, or weekly) sampling that can be used to address questions regarding the relationship of AMR with changing seasons, weather conditions, or to capture the impact of anthropogenic events such as pollutions incidents. We have broadly divided the sampling frequency into three categories:

1. High frequency sampler (more than once per month).
2. Medium frequency sampling (less than once per month).
3. Low frequency sampling (less than once per year).

2.3.3 Presence of an archive

Sample archives are a valuable resource for ecological and public health monitoring programmes (Dolfing & Feng, 2015; Tsangaras & Greenwood, 2012). Such archives, either of tissue, environmental samples (e.g., faecal samples) or of cultured microbes, allow re-investigation and analysis over longer periods. This retrospective analysis has the advantage of hindsight, for example, allowing the identification of the emergence of a particular disease-causing microorganism within a population. A high-profile example of this was retrospective analysis of wastewater in Italy, providing evidence that SARS-CoV-2 was circulating within populations in late 2019, rather than early 2020 as was first assumed from clinical evidence (La Rosa *et al*., 2021). There are two categories:

1. Archive present.
2. No archive present.

2.3.4 Interaction of wildlife target with humans

The interaction of wildlife target with other receptors (humans, plants or animals) is important, especially if the aim of the surveillance is to understand AMR exposure risk and disease transmission from the environment to humans and/or domestic animals/plants. Certain wildlife host species are associated with human health outcomes, due to their ability to transmit disease in their role as human food, their close association with human habitation or food production, or their role in parasitising or feeding on humans and animals. Examples of wild flora and fauna that are eaten include wild/semi-wild game species (e.g., waterfowl, grouse, pheasants, and deer) and plants (e.g., wild fruit, mushrooms, and salads). Examples of species with close associations with human habitation and or/food production include some rodents (e.g., mice and rats), or birds (e.g., house sparrows and pigeons). Species that feed/parasitise humans and domesticated animals and plants include invertebrates such as flies, fleas, mosquitos, and ticks, as well as a wide range of herbivorous invertebrates. We have included three categories to represent the relevance of the host species to human health, including:

1. Highly associated with humans.
2. Less frequently associated with humans.
3. Not associated with humans.

2.3.5 Relevance of the microorganism target to human health

AMR is largely an issue in humans and domesticated animals (e.g., pets and livestock) and plants (e.g., food crops), as these are the species treated with antimicrobials in response to microbial disease. As a result, surveillance of AMR in pathogenic microorganisms and genes that can infect and cause disease in these species have high relevance to human and fauna/flora health. However, AMR is a much wider issue, as resistance genes can transfer between pathogenic and non-pathogenic microbial strains and species via a range of genetic transfer mechanisms. As a result, understanding AMR in non-pathogenic, commensal microorganisms that are found in both wildlife and human/domestic host species

is relevant to understanding AMR dynamics. We constructed three categories to represent the relevance of the microorganism or AMR gene, including:

1. Known pathogen (animal, human or plant).
2. Known member of a host-associated microbiome.
3. Neither a pathogen nor a member of a host-associated microbiome.

2.3.6 Collection of AMR data

This category denotes whether the scheme currently collects data on AMR microorganisms or not. Schemes that currently collect AMR data are higher ranked than those that currently do not (but may have the potential to do so) due to the ability to retrospectively analyse samples. Schemes that currently do not are further subdivided into schemes that measure and collect data on microbes (for example via culturing or DNA analysis) versus those that currently do not but have the potential to do so. The three categories are:

1. AMR data collected.
2. AMR data not collected but microbial data is collected.
3. No AMR or microbial data collected.

The evaluation criteria across all six categories are summarised in Table 1 overleaf.

Table 1 Evaluation criteria used to assess existing wildlife surveillance schemes in England.

Score	Distribution and abundance	Frequency of sampling	Presence of a sample archive	Interaction of wildlife target with humans	Relevance of the microorganism target to human health	Collection of AMR data
1	National and abundant	High frequency sampler (more than once per month).	Yes	Eaten or highly associated with humans.	Known pathogen (animal, human, plant).	AMR data collected
2	National and low abundance	Medium frequency sampling (less than once per month).	No	Less frequently eaten or less frequently associated with humans.	Known member of a host-associated microbiome.	AMR data not collected but microbial data is collected
3	Regional and abundant	Low frequency sampling (less than once per year).		Not eaten or not associated with humans.	Neither a pathogen nor a member of a host-associated microbiome.	No AMR or microbial data collected.
4	Regional and low abundance					

2.4 Scheme selection process

A variety of wildlife surveillance schemes exist in the UK, covering a range of species, with different surveillance purposes and targets. There is currently no central database or source of information for UK wildlife surveillance activities, although some partnerships representing groups of different surveillance activities do exist (e.g., the Animal & Plant Health Agency's (APHA) Diseases of Wildlife Scheme http://apha.defra.gov.uk/vet-gateway/surveillance/seg/wildlife.htm). Our approach to scheme identification and collation of information was based on existing knowledge of schemes within both the UKCEH project team and Environment Agency project management team, contact with key UK organisations involved wildlife monitoring (e.g., the APHA), and wider internet searches.

Schemes were preferentially selected and contacted based on whether they collected:

- Information about wild flora and/or fauna in England.
- Physical samples of wild flora and/or fauna in England.
- Data on disease-causing organisms from wild flora and/or fauna.
- Data at a regional or a national level.

Twenty-six organisations and/or individuals were contacted to provide information about defined schemes, or to enquire about surveillance activities carried out by those organisations. An online form (https://forms.office.com/r/krxjVR1Fyy) was created to collect standardised information about existing schemes, using the questions below. Questions without defined options were free-text boxes. Where multiple choice options were provided, the "other" option allowed the submission of a free text answer.

Wildlife surveillance for AMR survey questions:

1. Please provide a brief description of the surveillance scheme.
2. What type of organism is targeted for surveillance?
    a. Mammal
    b. Bird
    c. Fish
    d. Reptile
    e. Invertebrate
    f. Plant
    g. Amphibian
    h. Other
3. If surveillance is targeted at a single or few species, please name them.
4. Are any species excluded from the surveillance scheme? (Answer NA if not relevant).
5. What habitat are they present in?
    a. Terrestrial
    b. Freshwater
    c. Marine
    d. Other

6. What is the purpose of the surveillance scheme (e.g., disease surveillance, population surveillance etc.)?
7. What type of samples are collected?
    a. Carcase
    b. Faecal
    c. Blood
    d. Tissue
    e. Other
8. On average, how many samples are collected/analysed per year?
9. What type of surveillance is performed (passive = *ad hoc* samples, active = targeted sampling)?
    a. Passive
    b. Active
    c. Other
10. Please provide details about how the samples are submitted. E.g., professional surveillance teams, research projects, rescue rehabilitation centres.
11. If microbiology data are collected, what methods are used?
    a. Culture based microbiology
    b. Bacterial antibiotic sensitivity testing
    c. Polymerase Chain Reaction (PCR)
    d. Quantitative PCR (qPCR)
    e. Metagenomics/Transcriptomics
    f. Other
12. If microbiology data are collected, what species (if any) are targeted?
13. If microbiology data are collected, are data on AMR collected?
    a. Yes
    b. No
14. If bacterial antibiotic sensitivity testing is performed, please provide more details. E.g., is this done on all samples submitted? If not, how are they selected? Which antimicrobials are tested against etc.
15. How long has the scheme been running for?
16. What is the geographic distribution of the scheme (e.g., local, regional, national)?
17. How frequently are samples collected?
18. Are samples archived, and if so, in what format and how far back does the archive extend?

2.5 Overview of participating wildlife surveillance schemes

A total of 13 schemes provided information on the scheme structure via the web form. An overview of the participating schemes is shown in Table 2 below.

Table 2 List of schemes that supplied information on surveillance activities.

Scheme	Scheme description	Organisation
Diseases of Wildlife scheme	Diseases of Wildlife scheme run by APHA and funded through Defra performs scanning surveillance in England and Wales of free-living vertebrates. It involves full diagnostic post-mortem exam and lab testing of eligible submissions (truly wild or in rehab facilities for less than 48 hours, no obvious trauma to explain death).	Animal and Plant health Agency (APHA)
Passive bat surveillance scheme	Passive bat surveillance scheme to test for lyssavirus infection	Animal and Plant health Agency (APHA)
Garden Wildlife Health (GWH)	Scanning disease surveillance for garden birds, amphibians, reptiles and hedgehogs across Great Britain (www.gardenwildlifehealth.org) Partners = Zoological Society of London, British Trust for Ornithology, Froglife and the Royal Society for the Protection of Birds.	Zoological Society of London (ZSL), the British Trust for Ornithology (BTO), Froglife and the Royal Society for the Protection of Birds (RSPB)
Rothamsted Insect Survey	The Insect Survey is host to a nationwide network of light-traps and suction-traps that collect invaluable data on the migration of moths, aphids and insect biodiversity more generally.	Rothamsted Research
Fish tissue archive	Collection and archiving of fish samples (mainly roach) from English rivers for the surveillance of bioaccumulated pollutants	UK Centre for Ecology & Hydrology (UKCEH)

Predatory Bird Monitoring Scheme (PBMS)	The Predatory Bird Monitoring Scheme (PBMS) monitors the residues of chemical contaminants in diurnal raptors and owls from the UK. As part of the PBMS we ask the public to submit birds that have been found dead to the scheme by post.	UK Centre for Ecology & Hydrology (UKCEH)
National Honey Monitoring Scheme (NHMS)	Surveillance of plant pollen and nectar analysed from honeybee honey samples using environmental DNA metabarcoding.	UK Centre for Ecology & Hydrology (UKCEH)
Fish disease surveillance	1. Disease surveillance during national incident response to understand the cause of fish kills in freshwater fisheries and prevent spread of high-risk diseases (bacteria routinely cultured). 2. Health check samples involving examination of 'healthy' fish for regulated pathogens or ill-health prior to fish movement (bacteria not sampled unless clinical disease present).	Environment Agency
Otter surveillance	Cardiff University Otter Project (School of Biosciences, Cardiff University) get shipped otters found dead from across the UK. We conduct post-mortems and archive a wide range of tissues and samples from each Otter.	Cardiff University
National Bat Monitoring Programme	National Bat Monitoring Programme. Volunteers carry out annual counts of bat at roosts and hibernacula and counts of activity levels (numbers of passes) in the field. One project involves trapping surveys.	Bat Conservation Trust (BCT)
Forest Research, TreeAlert	Specific surveillance schemes for particular tree pests and pathogens, general surveillance through TreeAlert	Forest Research
Tick Surveillance Scheme	The Tick Surveillance Scheme was set up in 2005 and is the only scheme that records tick distributions on a national scale. All	UK Health Security Agency (UK HSA)

	records are available on the National Biodiversity Network (NBN) gateway for research and public use.	
Nationwide mosquito surveillance project	A network of mosquito traps. By collecting mosquitoes HSA aim to understand the population dynamics and seasonality of mosquitoes at key habitats and across regions. The project collaborates with organisations across the country to run the mosquito traps.	UK Health Security Agency (UK HSA)

A summary of the major characteristics of the contributing wildlife surveillance schemes is provided in the section below. Based on answers to the free-text question, "What is the purpose of the surveillance scheme?", schemes were subsequently categorised into three main scheme types (although some schemes covered more than one purpose): 1) surveillance for chemical contaminants (usually bioaccumulating chemicals such as pesticides and metals); 2) wildlife disease surveillance; and 3) population surveillance to understand changes in the distributions and population numbers of wildlife (Figure 1).

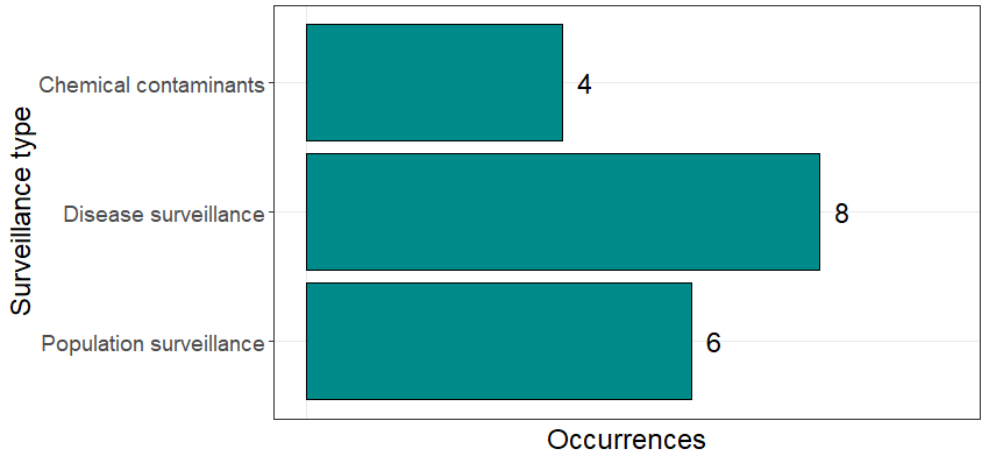

Figure 1 Main purpose of the wildlife surveillance schemes.

Mammals were the best represented group, covered by five schemes, followed by invertebrates (four), birds (three) and fish (two) (Figure 2). Only one monitoring scheme, managed by Forest Research, primarily targeted plant hosts of microbes. A second scheme, the National Honey Monitoring Scheme, targeted an invertebrate species, the European honeybee, *Apis mellifera*, as the primary host, but also collects data on the composition of plant pollen and nectar from honey samples. Wild plant monitoring schemes do exist in the UK, particularly those that utilise citizen scientists as the primary source of data (e.g., the National Plant Monitoring Scheme https://www.npms.org.uk), but these do not collect physical samples for analysis, so were excluded from this analysis.

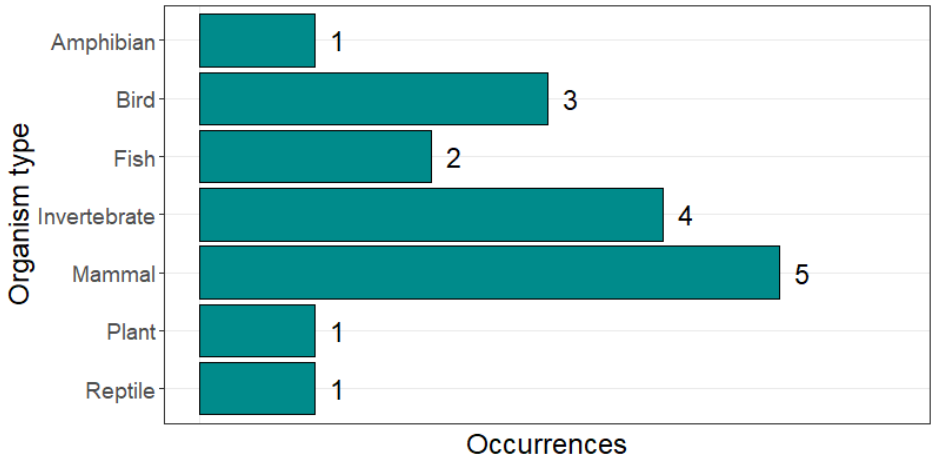

Figure 2 The number of instances where each group of organisms was the target of surveillance. More than one group can be present in a single surveillance scheme.

The species targeted in the wildlife surveillance schemes varied in scope. Several schemes focused on single species, for example: the National Honey Monitoring Scheme, which focusses on the European honeybee (*Apis mellifera*); the Cardiff University Otter Project, which focusses on the Eurasian otter (*Lutra lutra*); and the National Fish Tissue Archive, which primarily archives samples of roach (*Rutilus rutilus*). Other schemes focus on defined groups of organisms, such as: the Predatory Bird Monitoring Scheme (PBMS), which collects samples of any native diurnal raptor and owl species; and the National Bat Monitoring Programme which collects data on the distribution of most UK bat species. Finally, some wildlife surveillance schemes have a much wider remit, including: the APHA Diseases of Wildlife scheme, which covers a broad remit of diseased vertebrates, collected and submitted by partner agencies; the Rothamsted Insect Survey, which collects invertebrate species that are trapped using light-traps and suction-traps; and the Environment Agency's fish disease surveillance programme, which analyses disease in freshwater or estuarine/migratory fish species from any river, lake, canal, or pond.

For the target habitats, the schemes were mostly split between freshwater (seven) and terrestrial (nine) habitats, with only two schemes targeting species found partially in marine habitats (the Predatory Bird Monitoring Scheme and the Diseases of Wildlife scheme) (Figure 3).

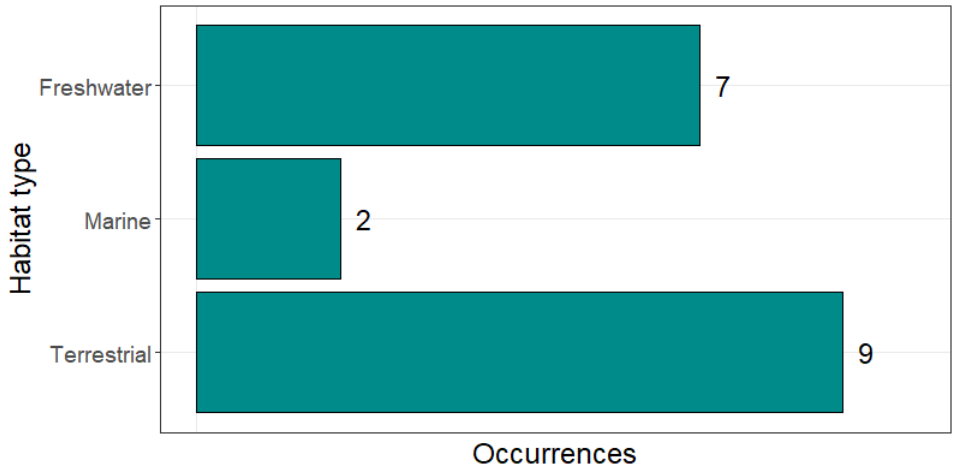

Figure 3 Habitats covered by the wildlife surveillance schemes. More than one habitat can be covered in a single surveillance scheme.

Animal carcases (individuals) were the most collected sample type (nine instances) followed by specific body tissues (five instances), such as dissected kidneys and livers, as well as blood samples (two instances) in both the Cardiff University Otter Surveillance Scheme and the Environment Agency's fish disease surveillance programme (Figure 4). Although all schemes collected samples, this was not the focus in all cases. For example, in the National Bat Monitoring Programme, faecal samples have been collected for discrete projects, but this is not currently done in a widespread or systematic manner.

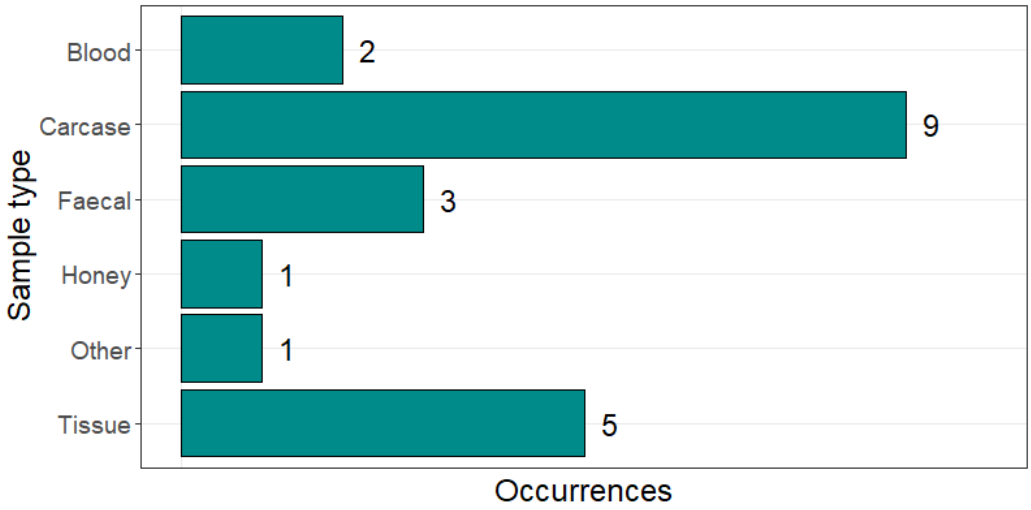

Figure 4 Sample types collected

Eight of the 13 wildlife surveillance schemes either have or currently collect information on microbiology parameters (Figure 5). Of these, both culture-based microbiology and analysis by polymerase chain reaction (PCR) were the most frequently applied methods, with five instances each. Only the APHA's Diseases of Wildlife scheme routinely performs analysis of AMR, using a combination of bacterial Antibiotic Susceptibility Testing (AST), PCR and metagenomics. The Cardiff University Otter Surveillance Scheme is currently performing pilot analysis on AMR using both culture-based microbiology (targeting *Escherichia coli*) and

metagenomics on otter faecal-rectal samples. The APHA's passive bat surveillance scheme is the only scheme to routinely target a single species of microbe (the Rabies virus – *Lyssavirus*). Both the APHA Diseases of Wildlife scheme and the Environment Agency's fish disease surveillance programme perform investigative analysis to identify disease-causative microorganisms.

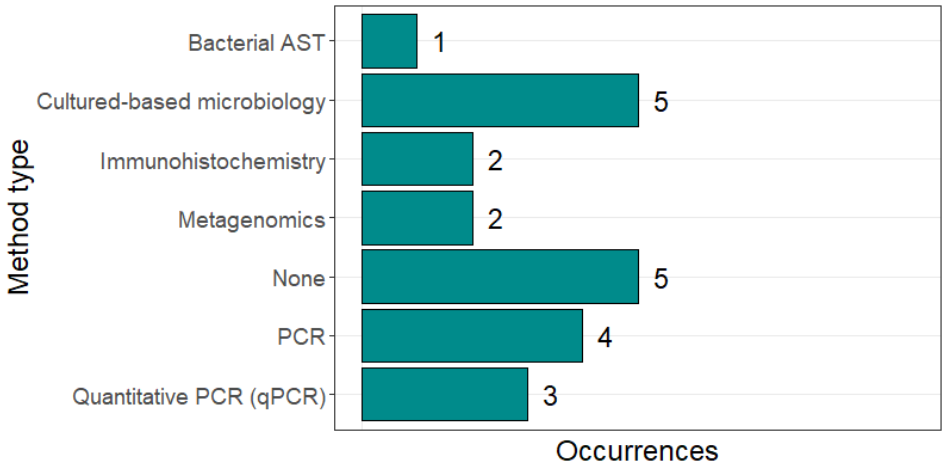

Figure 5 Methods used to analyse microbiology in wildlife surveillance schemes.

2.6 Evaluation of monitoring schemes in England

The outcome of the evaluation criteria for the 13 participating wildlife surveillance schemes is shown in Table 3. As stated previously, their suitability for wildlife AMR surveillance is context-dependent, determined by the research or surveillance aims. However, our evaluation criteria can be used as a broad assessment of the current and future potential of a scheme to address questions in AMR and wildlife.

Most schemes targeted organisms that are abundant and are distributed nationally. We acknowledge that there is an inherent bias in our scheme selection procedure that means that larger schemes that target nationally distributed and abundant organisms were more likely to be identified and included in this review. Two schemes, the Predatory Bird Monitoring Scheme and the Cardiff University Otter Surveillance scheme target organisms that are inherently less abundant due to their trophic position as predators, but both groups of organisms are distributed nationally.

The schemes were a mix of both active and passive surveillance, representing six exclusively passive schemes, three exclusively active schemes and four schemes that use a mix of both active and passive surveillance. It was notable that there were only two schemes that deployed active surveillance that collected microbial data (the Environment Agency's Fish Disease surveillance and Forest Research's plant health surveillance), and there were no active surveillance schemes that collected data on AMR.

Most schemes had some form of sample archive, with only the Bat Conservation Trust's National Bat Monitoring Programme not having any archived material. Where information

was provided, the archives comprised of microbial cultures (e.g., the APHA Diseases of Wildlife scheme and the Garden Wildlife Health scheme), tissues (Predatory Bird Monitoring Scheme, Diseases of Wildlife scheme, Garden Wildlife Health, Lyssavirus bat surveillance scheme, and the National Fish Tissue Archive), whole organisms (Insect Survey), faecal-rectal samples (Cardiff University Otter Surveillance) and honey (National Honey Monitoring Scheme).

Two schemes scored highly in terms of their evaluation criteria, which were the APHA Diseases of Wildlife scheme and the Garden Wildlife Health scheme, both of which have wildlife disease and pathogens as their focus. Due to their focus on disease causing organisms, both collect microbiological data and, in the case of the Diseases of Wildlife scheme routinely collect data on AMR on microbial species considered to be veterinary pathogens. The Garden Wildlife Health scheme collects phenotypic and genotypic AMR data for specific studies rather than routinely.

Table 3 Evaluation of wildlife surveillance schemes in England and the UK. Numbers denote the evaluation criteria, where lower numbers are more highly ranked.

Scheme	Habitat	Distribution and abundance	Passive or active	Presence of a sample archive	Interaction of wildlife target with humans	Relevance of microorganism target to human health	Collection of AMR data
Diseases of Wildlife scheme (APHA)	Terrestrial; Freshwater; Marine;	National and abundant (1)	Passive	Yes (1)	Highly associated with humans (1)	Known pathogen (animal, human, plant) (1)	AMR data collected (1)
Garden wildlife health (ZSL)	Terrestrial; Freshwater; Marine;	National and abundant (1)	Passive	Yes (1)	Highly associated with humans (1)	Known pathogen (animal, human, plant) (1)	AMR data collected (1)
Lyssavirus bat surveillance scheme (APHA)	Terrestrial	National and abundant (1)	Passive	Yes (1)	Less frequently associated with humans (2)	Known pathogen (animal, human, plant) (1)	AMR data not collected but microbial data is collected (2)
National Bat Monitoring Programme (BCT)	Terrestrial	National and abundant (1)	Active	No (2)	Less frequently associated with humans (2)	NA	AMR data not collected but microbial data is collected (2)
Predatory Bird Monitoring Scheme (PBMS)	Terrestrial; Freshwater; Marine	National and low abundance (2)	Passive	Yes (1)	Less frequently associated with humans (2)	NA	No AMR or microbial data collected (3)

Cardiff University Otter surveillance scheme (CU)	Terrestrial; Freshwater	National and low abundance (2)	Passive	Yes (1)	Less frequently associated with humans (2)	Known pathogen (animal, human, plant) (1)	AMR data collected (1)
National Honey Monitoring Scheme (UKCEH)	Terrestrial	National and abundant (1)	Active	Yes (1)	Highly associated with humans (1)	NA	No AMR or microbial data collected (3)
Fish disease surveillance (EA)	Freshwater	National and abundant (1)	Active and passive	Yes (1)	Less frequently associated with humans (2)	Known pathogen (animal, human, plant) (1)	AMR data not collected but microbial data is collected (2)
Fish tissue archive (UKCEH)	Freshwater	National and abundant (1)	Active	Yes (1)	Less frequently associated with humans (2)	NA	No AMR or microbial data collected (3)
Forest Research, TreeAlert	Terrestrial	National and abundant (1)	Active and passive	Yes (1)	Less frequently associated with humans (2)	Known pathogen (animal, human, plant) (1)	AMR data not collected but microbial data is collected (2)
Tick Surveillance Scheme	Terrestrial	National and abundant (1)	Passive	Yes (1)	Highly associated with humans (1)	Known pathogen (animal, human, plant) (1)	No AMR or microbial data collected (3)

UK HSA Mosquito surveillance	Terrestrial; Freshwater	National and abundant (1)	Active and passive	Yes (1)	Highly associated with humans (1)	Known pathogen (animal, human, plant) (1)	AMR data not collected but microbial data is collected (2)
Insect Survey (Rothamsted Research)	Terrestrial	National and abundant (1)	Active and passive	Yes (1)	Less frequently associated with humans (2)	NA	No AMR or microbial data collected.

3 Current knowledge of AMR in wild fauna and flora

Recent decades have seen an increase in studies investigating the presence of AMR in non-human, wild organisms, for example, in crops, livestock, pets, and wild animals and plants. Research has found that microbiomes of wild organisms respond to and reflect the surrounding environmental pressures and could potentially act as both sinks and sources of AMR (Gwenzi et al., 2021). The role of wild organisms in the dissemination and persistence of AMR highlights their potential as a tool for monitoring AMR in the environment.

As previously discussed, the choice of species, method and endpoint for surveillance efforts often depends on the (research) question being asked. To gain an understanding of both the hosts and microbes targeted, methods used, and rationale given in the literature related to surveillance of wildlife for AMR, a semi-systematic, albeit non-exhaustive, rapid evidence review-style approach was undertaken.

3.1 Review methodology

3.1.1 Literature searching

Literature searches were conducted within the Web of Science (WoS) and Google Scholar publication search engines. WoS is one of the largest and most comprehensive bibliometric databases, containing articles from over 21,000 journals (Pranckutė, 2021; Web of Science). Due to the ability to perform advanced searches in WoS, searches were conducted using a single search string, as follows: 'AMR OR "antimicrobial resistance" OR "antifungal resistance" AND "Wild plants" OR "Wild Animals" OR Wildlife' (see here for the saved query weblink). WoS searches were restricted to the "Topic" field, which searches titles, abstracts, authors, keywords and KeyWords Plus. To avoid missing relevant publications not contained in the WoS database, the searches were supplemented with Google Scholar searches. Google Scholar searches included the keywords: antimicrobial, antibiotic, antifungal or AMR, resistance or resistant, wildlife or wild, and animal, plant, or flora. Backward citation chasing was undertaken to identify further relevant publications, although not in a fully systematic manner. Additional attention was devoted to plant-based and antifungal-focused articles using Google Scholar, as these were underrepresented in initial search results. All search results are up to date as of 26th October 2022, after which point no more searches were performed.

3.1.2 Literature screening

WoS search results were fully screened at title and abstract level, and duplicates were removed. The most relevant Google Scholar results were screened for inclusion by title and abstract, however not exhaustively due to the volume of results and time limitations. Only

publications focusing on wild animals or plants were included in the database, which excluded publications focusing on food-producing animals (e.g., livestock and aquaculture) and plants (e.g., food crops and fruit trees), companion animals, and captive animals (e.g., those in zoos, zoological collections, and semi-managed populations in reserves). However, wild animals in rehabilitation or rescue centres were still included. Some publications included both wild and non-wild animals and plants, thus the wild animal data were extracted and included in the database. In addition, some relevant publications were not included in the database if full texts were inaccessible, however, this was rare (n < 10 from WoS).

3.1.3 Database creation

Data from all relevant search results were extracted and collated to create a database of publications focusing on AMR in wildlife. The extracted variables included summary data, (e.g., authors, publication date and study location), methodological data (e.g., type of AMR assessed, analysis used, sample taken, and host, microbe and gene of interest), and rationale behind the study.

The country of study (i.e., where samples originated from, not the country the authors were based in) was extracted. This was seen as the more relevant location information, as it allows comparisons to UK habitats, wildlife, and socioeconomic factors.

Specifically, database column headings were as follows:

- Database ID,
- Title,
- Published date,
- Citation (including author(s), date, journal, etc.),
- Study location (by country),
- AMR type (categories – Antibacterial or Antifungal),
- Organism type 1 (main categories – Animal or Plant),
- Organism type 2 (subcategories – Amphibian, Bird, Fish, Invertebrate, Mammal, Mollusc, Plant, Reptile),
- Organism 3 (host species),
- Environment type (categories – Coastal, Freshwater, Marine, Terrestrial),
- Sample (physical sample type, for example, faeces or oral swabs),
- Method type 1 (main categories – Culture-based or Molecular-based),
- Method type 2 (sample analysis type, for example, antibiotic susceptibility testing (AST) or quantitative polymerase chain reaction (qPCR). Only AMR methods were included),
- Culture target (microbes targeted for culture-based methods),
- Molecular target (antimicrobial resistance genes targeted for molecular-based methods),
- Rationale (categories – Dissemination of AMR in the environment, Human health/Exposure to AMR, Spillover to the environment from anthropogenic contact, Testing methods or Other), and

- Do (some/all) the study species exist in the UK (categories – Yes or No).

3.1.4 Data analyses

On completion of the database, data were analysed for trends in country, region, and environment type of study, publication date, analysis methods and sample types, culture and molecular targets, host species, and study rationale. Data analyses were performed in Microsoft Excel and R, and figures were created using the "ggplot2" package in R (Wickham 2016) and ArcGIS v10.6.1 software (ESRI, 2022).

Host species were extracted from each publication and categorised taxonomically. Often, multiple host species appeared in each publication, therefore, the total number of occurrences for each taxon were counted within the database. Where lower taxonomic information was not given in the publication, taxa were recorded to the next best taxonomic level. For analyses, host organisms were grouped taxonomically at order level. However, some organisms were grouped at other taxonomic levels to better extract trends from the data. For example, the large order Artiodactyla (the even-toed ungulates) was split by the infraorder Cetacea (whales, etc.), and families such as *Bovidae* (cattle, etc.), *Cervidae* (deer, etc.), *Suidae* (pigs, etc.), *Camelidae* (camels, etc.) and more. Another example was the large order Carnivora that was the split to infraorder level Viverroidea (civets, mongooses, hyenas etc.hyenas, etc.), and families such as *Canidae* (dogs, etc.), *Ursidae* (bears, etc.), *Mustelidae* (weasels, etc.), *Felidae* (cats, etc.) and more. This was also done for other organism types, such as birds, for the diverse order Charadriiformes, which was split into the suborders Charadrii (wading shorebirds) and Lari (gulls, etc.).

Culture targets were extracted from each publication and categorised taxonomically to order, family, genera and even species level, whereas molecular targets (i.e., resistance genes) were extracted from publications and categorised according to antimicrobial class. As with host species, multiple culture and molecular targets appeared in each publication, therefore, the total number of occurrences for each culture taxa or resistance gene were counted within the database.

3.2 Review findings

Following WoS searches, a total of 550 publications were screened at title and abstract level. To supplement this, the most relevant Google Scholar results were also screened for inclusion in the database. The final database comprised a total of 453 publications, which were included in the following analyses. The database can be found in Appendix 1.

3.2.1 Temporal and spatial analyses

The year of publication was recorded for each paper included in the database. Publication years ranged from 1983-2022, with a general upwards trend in publication numbers over time and over 50% of publications occurring since 2019 (Figure 6). This recent increase in

publications relating to AMR in wild animals and plants reflects the growing interest, awareness and realisation of the importance of environmental AMR in a One Health context.

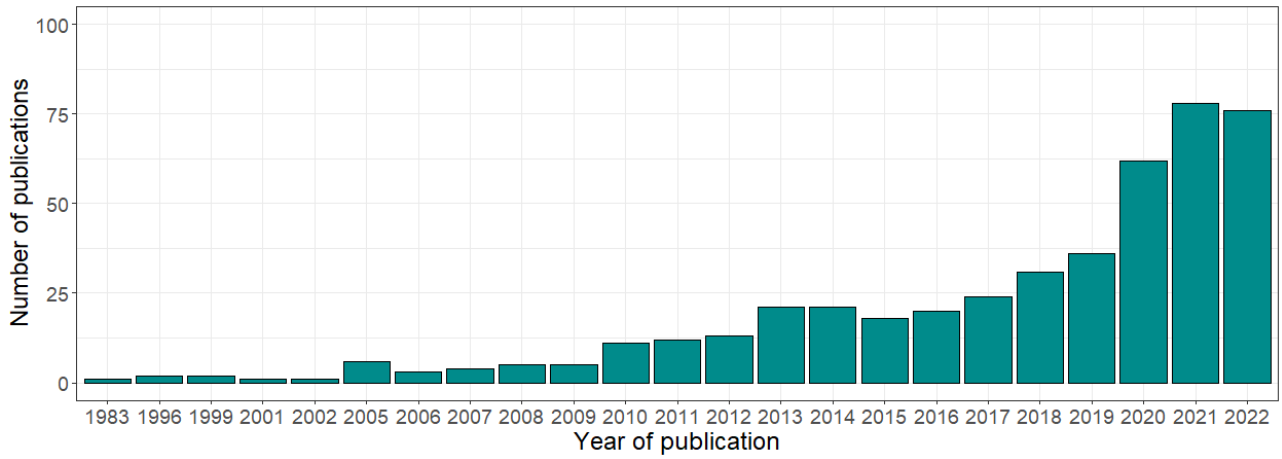

Figure 6 Temporal spread of publications investigating AMR in wild animals and plants.

A total of 85 study countries represents the publications in the database, belonging to seven continents, as follows: Africa (number of countries included = 22), Antarctica (n = 1), Asia (n = 16), Australia (n = 1), Europe (n = 30), North America (n = 10) and South America (n = 5).

The top five most prolific countries of study were Spain (number of publications = 51), Portugal (n = 45), the USA (n = 45), Brazil (n = 35) and Italy (n = 32). England was the 11th most studied country, accounting for ten publications in the database. Of the top 20 most studied countries, the highest proportion of studies were European, followed by North America. The number of publications per continent were as follows: Europe (n = 232), North America (n = 80), South America (n = 53), Africa (n = 47), Asia (n = 40), Australia (n = 21), and Antarctica (n = 6) (Figure 7).

Notably, although certain countries may have higher numbers of publications attributed to them, this can often be an overrepresentation from some research groups. For example, a research group with collaborators from Spain and Portugal authored many of the Spanish- and Portuguese-based studies. This overrepresentation can lead to duplicate samples in the database, even though they are included in multiple publications. An example of this is seen in the study of faecal samples from 181 ungulates from Portugal, which are analysed in both Torres et al. (2021) (focusing on the presence of colistin resistance in *Escherichia coli*) and Torres et al. (2022) (focusing on extended-spectrum β-lactamases (ESBL)-producing *Enterobacterales*). Another example from these collaborators is the study of 237 Iberian Wolf faecal samples analysed in Gonçalves et al. (2011) (focusing on vancomycin-resistant enterococci), Gonçalves et al. (2012) (focusing on ESBL-producing *E. coli*) and Gonçalves et al. (2013) (focusing more generally on AMR in enterococci and *E. coli*). Our results are also supported by this Spanish/Portuguese collaboration, which published a

bibliometric analysis of research concerning AMR in wildlife, which found themselves and their Spanish and Portuguese institutions, among the most productive in terms of number of publications (Torres et al., 2020). Although large-scale studies are often split into smaller stories for output publications, this is something to take into consideration when interrogating the database here. This biasing of the database is also likely to affect such metrics as most studied host organism, microorganisms, and genes.

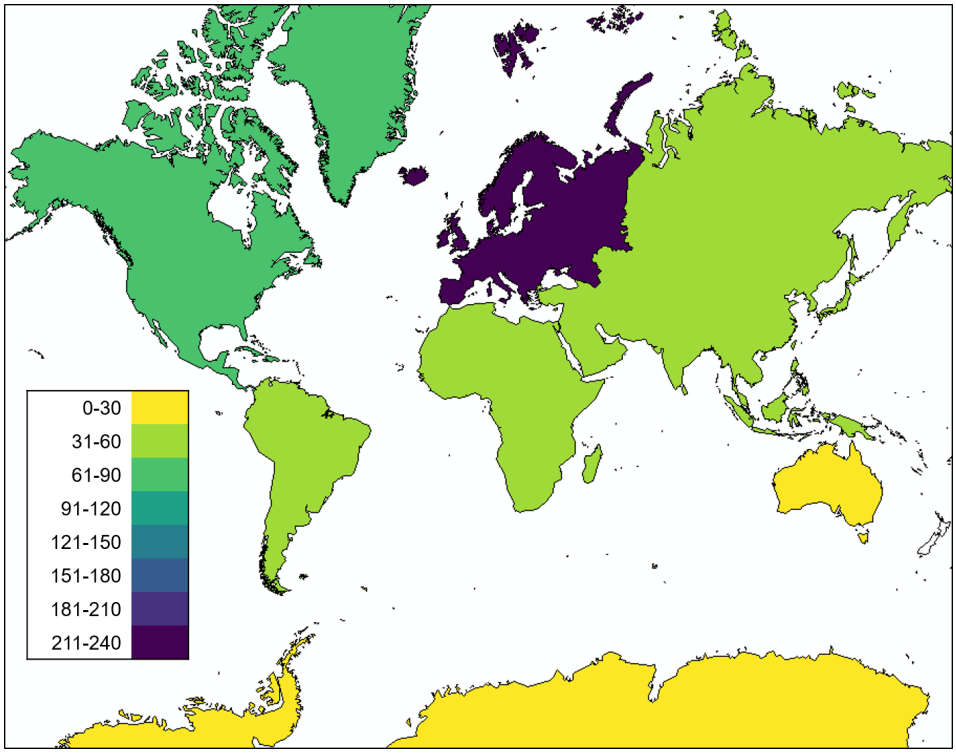

Figure 7 The number of publications investigating AMR in wild animals and plants per continent. Europe (n = 232), North America (n = 80), South America (n = 53), Africa (n = 47), Asia (n = 40), Australia (n = 21), and Antarctica (n = 6).

3.2.2 Targeted host taxa

In summary, the host taxa targeted throughout the literature mostly inhabited terrestrial environments, followed by coastal environments, with the number of publications sampling terrestrial and coastal environments being 369 and 86, respectively (Figure 8). The environment type least targeted in the literature was the marine environment (Figure 8). When the target host organism was unknown or lacking sufficient detail (i.e., only information on a high taxonomic level was given), the environment was recorded as “unknown”.

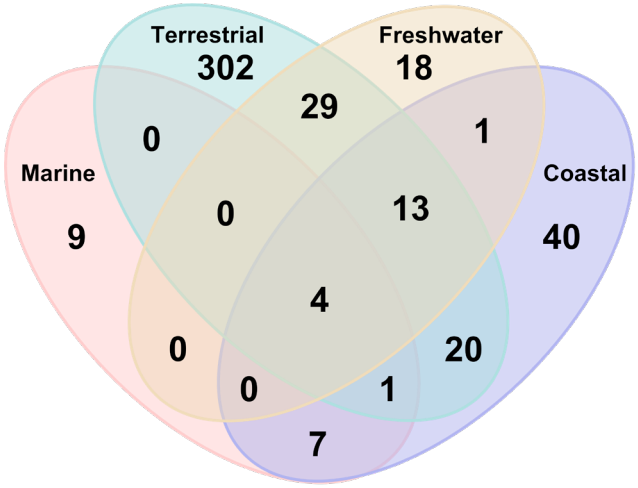

Figure 8 Number of publications studying each environment type. Number of publications with environment unknown = 9 (not shown).

The most targeted host taxa throughout the literature on AMR in wildlife were mammals and birds (Figure 9). Of the 453 publications in the database, 251 targeted mammals, 198 targeted birds, 38 targeted reptiles, 33 targeted invertebrates, 12 targeted fish, six targeted amphibians and five targeted plants. This made mammals and birds between 30-50 times more likely to be targeted than amphibians or plants. Of all publications included in the database, 65% contained wildlife than can be found in the UK.

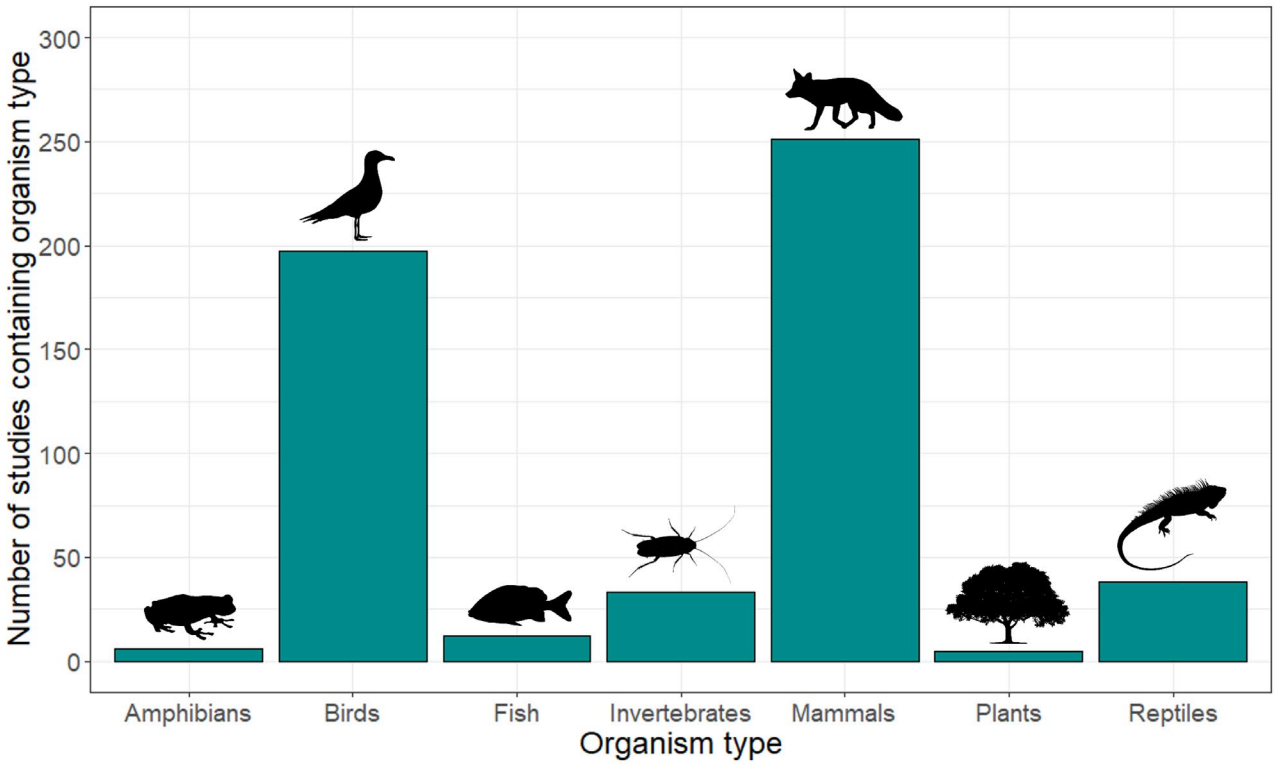

Figure 9 Number of publications investigating AMR in wild animals and plants per host taxa type.

The popularity of mammals as a target host taxon is likely due to several factors, including their genetic relatedness and shared evolutionary history with humans, likelihood of carrying similar pathogens and suffering from similar diseases, proximity to urban centres and anthropogenic activities, and ease of sampling/capture. Of the mammals, the order Artiodactyla (the even-toed ungulates) was sampled most frequently (number of occurrences = 259) (Figure 10). The most studied families within Artiodactyla were Cervidae (e.g., deer and relatives) (n = 90), Bovidae (e.g., cattle) (n = 76) and Suidae (e.g., pigs and relatives) (n = 73). Notably, the wild boar (an ungulate in the Suidae family) was the most studied species in the whole database, with 62 occurrences. As previously mentioned, certain species and countries of study are overrepresented in the database due to prolific publishing from large collaborative research groups. Wild boars and deer are examples of these overrepresented groups. Ungulates such as these overlap with humans and anthropogenic activities by means of contact with livestock and as a target of hunting. Combined with their large population numbers and wide distribution within the environment, this makes them desirable species for AMR surveillance.

Mammals within the order Carnivora were the second most studied throughout the literature, with *Mustelidae* (e.g., Weasels, badgers and otters) (n = 70) and *Canidae* (e.g., dogs, foxes and wolves) (n = 69) the most studied families. Rodentia (e.g., rats, mice, squirrels, beavers, etc.) were the third most studied order (n = 138). Wild carnivores and rodents are known to be a reservoir of many zoonotic diseases (Han et al., 2015). Rodents often comfortably inhabit urban areas and unlike most other wild animals, even human households (Gwenzi et al., 2021). Their vast numbers and distribution in most environments, and their potential to disseminate and transfer AMR to humans through direct contact, food, companion animals, and environmental and household matrices, (Gwenzi et al., 2021), make them a priority species of interest for AMR surveillance.

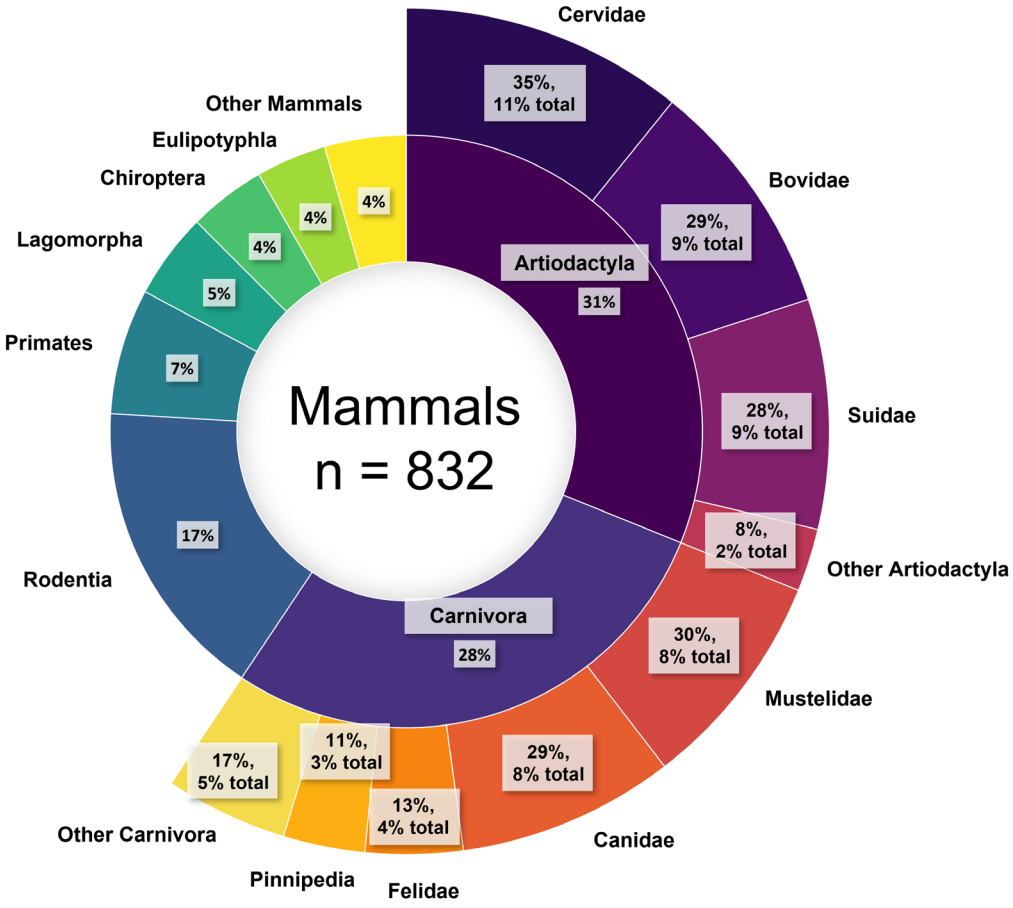

Figure 10 Proportion of different types of mammals, split by taxonomic group (order or family). Numbers indicate percentage of total mammal occurrences (total number of specific mammal occurrences = 832, within 251 mammal publications).

The second most studied target host group throughout the literature was birds (Figure 9). This popularity in study is likely due to their large population numbers, ability to migrate widely, cohabitation of urban centres and proximity to anthropogenic activities (such as agricultural, landfill, and wastewater treatment sites). Birds can act as a widely disseminating reservoir of human disease and have been suggested to be a disseminator of AMR (Ahmed et al., 2019; Nabil et al., 2020). This may be particularly true for birds that undergo vast migrations, such as geese and gulls, which have also been shown to carry clinically-relevant AMR (e.g., Ahlstrom et al., 2018; Jarma et al., 2021; Navedo et al., 2021) However, such movements can make it more challenging to link the drivers of AMR with host-associated AMR due to the number of habitats each organisms is exposed to. Almost a third of the birds occurring in the literature were of the order Passeriformes (passerines) (330 occurrences in the database) (Figure 11), which is the most species-rich order of birds, containing over half of all bird species (Ricklefs, 2003; Ricklefs, 2012). Passerines are considered the “perching birds” (e.g., sparrows, finches, thrushes, and corvids). Of the passerines studied, the most targeted were those in the *Corvidae* family (e.g., crows, rooks, magpies, and ravens), with 66 occurrences in the database. Corvids are known to be important hosts of some zoonotic diseases, and in some cases have been used to predict

human disease outbreaks (e.g., West Nile Virus (David et al., 2007)). This known carriage of human diseases and existing use as a surveillance tool (albeit using carcases), highlights their potential as a surveillance tool for monitoring environmental AMR.

The second most well-studied bird order was the Accipitriformes (e.g., hawks, eagles, vultures, and kites) (144 occurrences in the database), the third most studied order was Charadriiformes (e.g., gulls and auks) (with the majority (72%, n = 101) of these being within the Lari suborder, which contains gulls and terns), and the fourth most studied order was Anseriformes (e.g., ducks, geese, and swans) (85 occurrences in the database) (Figure 11).

Birds of prey, such as the Accipitriformes (e.g., hawks and eagles), Falconiformes (e.g., falcons and kestrels) and Strigiformes (e.g., owls), play a vital role in the food chain and are often the apex predators in their food webs. They are mostly carnivorous and opportunistic in feeding habits, feeding on smaller birds, rodents, reptiles, and insects (Sonerud *et al*., 2014). Therefore, they may represent an integrated and much broader measure of AMR across wild species. Birds of prey also have the potential to feed on animals that have been treated with antibiotics, e.g., pheasants, thus representing a link between anthropogenic antimicrobial usage and the environment.

A focus on gulls (i.e., those in the suborder Lari) and their relatives within the literature is likely due to their ubiquity in the environment and cohabitation with humans in urban centres. Similarly to rodents, such as rats, gulls have the ability to occupy a variety of habitats, both coastal and inland, on farmland, in towns and cities, and frequenting landfill and wastewater treatment sites (Coulson, 2015). This can also be true for corvids and pigeons. The relationship with human-inhabited environments enables gulls to be useful targets to assess spillover from anthropogenic activities into wildlife, particularly concerning AMR, as gulls can encounter highly polluted environments.

Similarly to gulls, waterfowl, such as the Anseriformes (e.g., ducks, geese, and swans), can come into contact with polluted environments. Aquatic environments are often the sinks of much anthropogenic pollution, such as agricultural runoff, treated and untreated wastewater, and aquaculture. Waterways not only act as a reservoir of persistent chemical pollution and resistant microorganisms, but also as one of the most significant sources and means of dissemination of AMR throughout the environment (Taylor et al., 2011, Cabello et al., 2016). Therefore, waterfowl that inhabit contaminated water sources, are likely to also be reservoirs and transmission routes of AMR, making them desirable candidates for the surveillance of AMR in the environment.

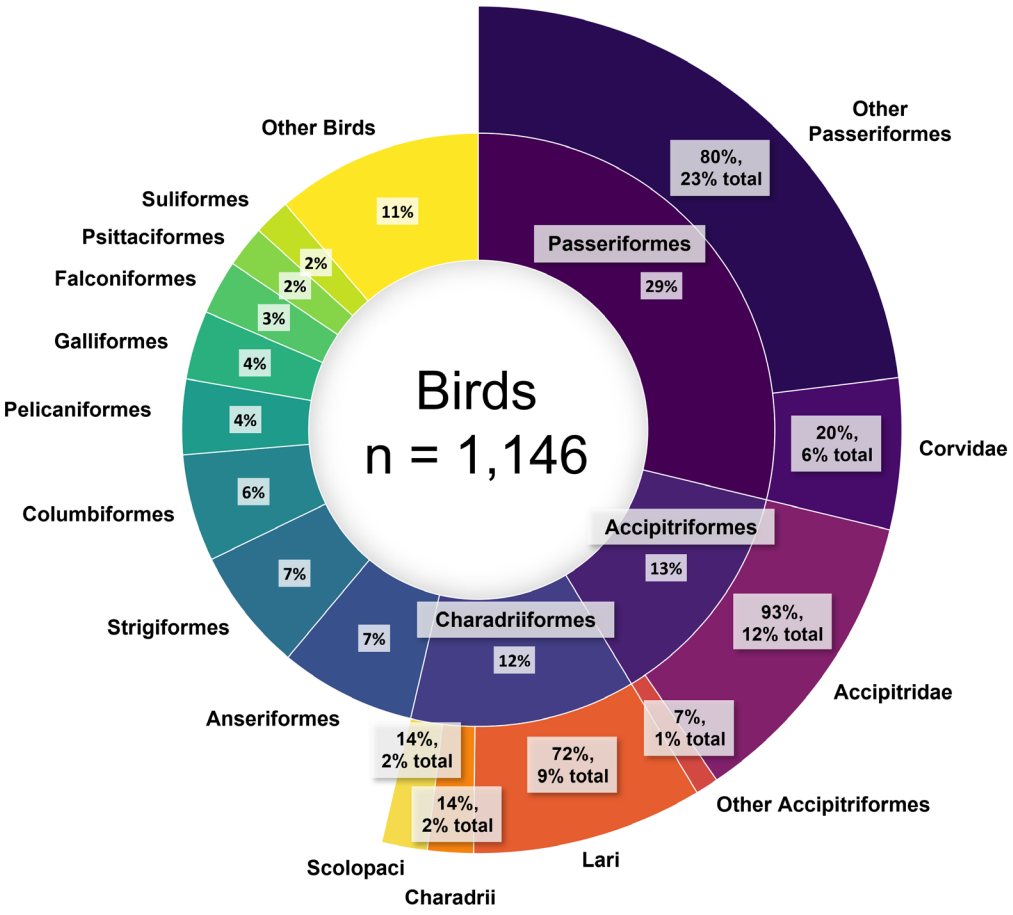

Figure 11 Proportion of different types of birds. Numbers indicate percentage of total bird occurrences (total number of specific bird occurrences = 1,146, within 198 bird publications).

Amphibians, reptiles, invertebrates, and fish were all less studied than mammals and birds, with the number of publications focusing on them all in total being less than half of those focused on birds (Figure 9). Following mammals and birds, reptiles were the next most popular host taxa (Figure 12b).

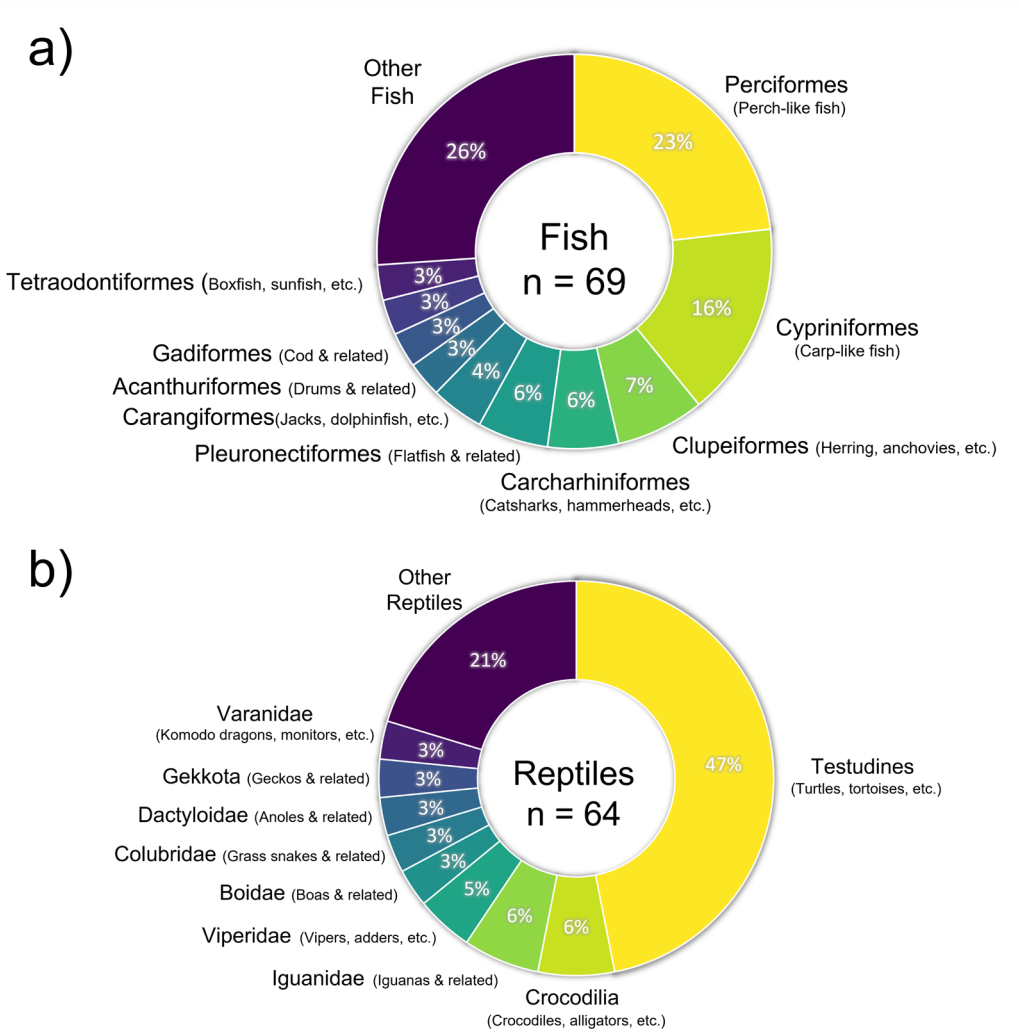

Figure 12 Proportion of different types of a) fish and b) reptiles. Numbers indicate percentage of total occurrences (total number of specific fish occurrences = 69, within 12 fish publications; total number of specific reptile occurrences = 64, within 38 reptile publications).

Overall, although the number of publications including the lesser-studied organisms may have been lower, some of the specific taxonomic groups had relatively high occurrences, for example, the orders Testudines (e.g., turtles and tortoises) and Diptera (e.g., flies), had 30 and 31 occurrences in the database, respectively (Figure 12b and Figure 13a). The most the most popular taxonomic host groups for each organism type were as follows: for fish – the order Perciformes (perch-like fish) (16 occurrences in the database) (Figure 12a), for reptiles – the order Testudines (e.g., turtles and tortoises) (30 occurrences in the database) (Figure 12b), for invertebrates – the order Diptera (e.g., flies) (31 occurrences in the database) (Figure 13a), and for amphibians – the orders Urodela (e.g., newts and salamanders) and Anura (e.g., frogs and toads) (both with 4 occurrences each in the database) (Figure 13b).

Throughout the literature, there was a noticeable omission of publications focusing on plant-based wildlife (n = 5) (Figure 9). Publications sampling plants targeted fruit plants, conifers,

woody landscape plants, eucalyptus trees and forest plants (Figure 13c). Notably, one study included in the database aimed to use conifer needle phyllosphere (portion of plant above ground) as a passive sampler of bioaerosolised ARGs (George et al., 2022).

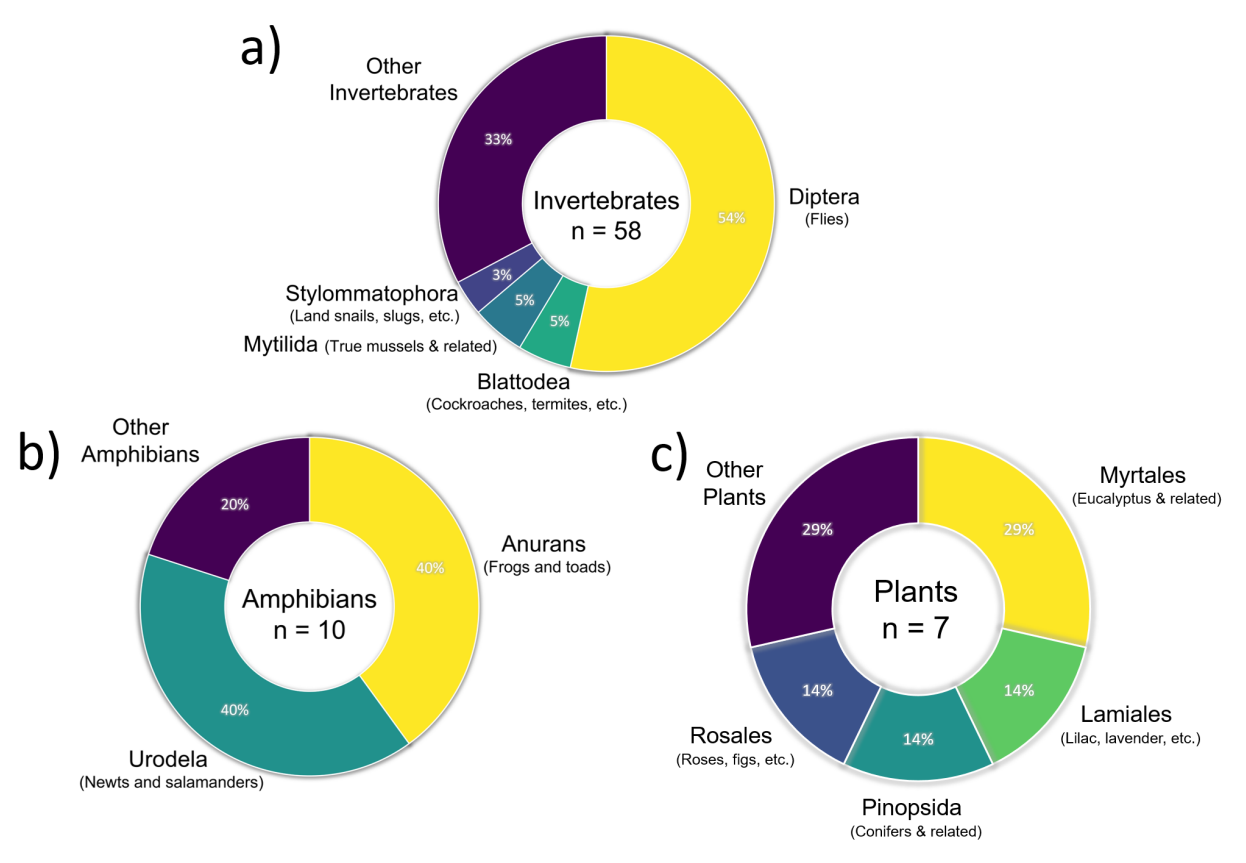

Figure 13 Proportion of different types of a) invertebrates, b) amphibians and c) plants. Numbers indicate percentage of total occurrences (total number of specific invetebrate occurrences = 58, within 33 invetebrate publications; total number of specific amphibian occurrences = 10, within 6 amphibian publications; total number of specific plant occurrences = 7, within 5 plant publications).

3.2.3 Analysis of methods used

To evaluate the methods used and targets of interest throughout the literature surrounding AMR in wildlife, details were extracted from publications on AMR type, culture and/or molecular-based approach, AMR methods used, microorganism targeted (culture target) and ARG/other gene of choice (molecular target).

In summary, of the 453 publications collated in the database, the vast majority were based on bacteria, i.e., antibiotic resistance (n = 433). As previously mentioned, extra effort was allocated to identifying publications focusing on antifungal resistance, to gain a broader

insight into surveillance efforts of AMR in wildlife, yet only 18 publications based on fungi were included in the database.

3.2.3.1 Sample types

Sample type is often dependent on the question of study, i.e., is the study aiming to look at AMR carriage in specific zoonotic pathogens? Or is the study wanting to get as much microbial DNA as possible for molecular analyses of thousands of resistance genes? Sample type is also constrained by the target host organism, type of habitat and the time, expertise and budget of the researchers involved. The samples most collected in the literature were faeces and cloacal/rectal/faecal swabs (Figure 14). The greater focus on faecal matter and the gut microbiome of the organisms sampled is likely due to many things, including the fact that many microbial pathogens exist in the gut, such as enteric bacteria like *E. coli*, *Salmonella spp*. and *Klebsiella spp*., and that the vast majority of an organisms' microbiome is found in the gut (Quigley, 2013). Environments such as the human or animal gut microbiome are not only thought of as reservoirs of AMR, but are also considered to act as reaction vessels, where selection for and HGT of ARGs may occur (Eckert et al. 2016; Kent et al. 2020). Ease of sampling is also likely to be factor in the popularity of this sample type, as collecting faeces is a relatively easy, and largely non-invasive and non-destructive sampling method. Other common sample types included nasal, oral and oesophageal swabs, which also represent a niche of pathogens (e.g., *Staphylococcus spp.*) and non-destructive sampling. Choosing the appropriate sample type for an AMR surveillance campaign is dependent on both the study question and the constraints surrounding the study (e.g., time, environmental, expertise, and budget). The gut microbiome of wild organisms represents the effects of chronic exposure to resistance driving chemicals in the environment, and thus is likely an integrated measure of the prevalence of AMR more broadly in the environment.

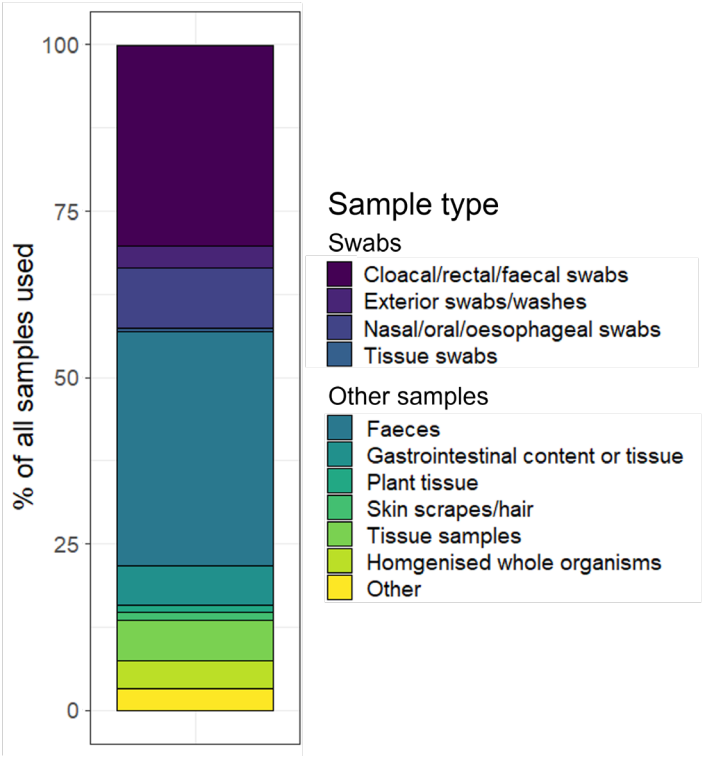

Figure 14 Proportion of different sample types collected in studies found in the literature. Proportion shown as total percentage of all sample occurrences (total number of sample occurrences = 550).

3.2.3.2 AMR-related analysis methods

Most publications in the database (76%) covered both culture- and molecular-based approaches (Figure 15). Frequently, these were studies that isolated bacteria (often *E. coli*) and performed AST, whilst also extracting DNA and running PCRs for ARGs.

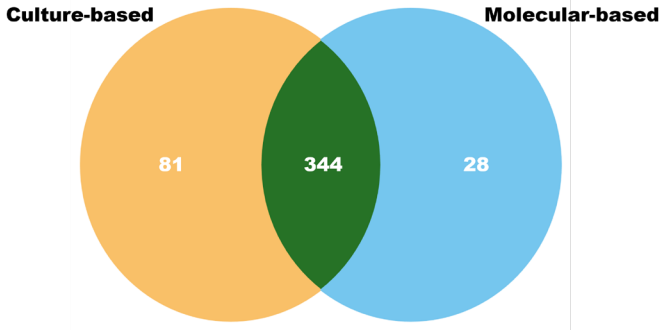

Figure 15 Number of publications that were culture-based, molecular-based or both.

Similar to all other decisions surrounding AMR surveillance, the choice of analysis type is often dictated by the research question. The most used sample analysis type found in the literature collated here was using antibiotic/antifungal susceptibility testing (AST) (Figure 16), which allows for a broader look at phenotypic resistance traits of cultured isolates. The second most used sample analysis technique was PCR, which allows the assessment of the qualitative diversity of resistance genes among samples using a presence/absence-based screen of resistance genes. However, both AST and PCR have their disadvantages, for example, AST relies on only culturable microorganisms, which are a small fraction of all microorganisms, and PCR requires the *a priori* selection of gene targets.

Other, less popular sample analysis methods were identified in the literature (Figure 16). For example, the use of qPCR allows for the measurement of quantitative changes in prevalence of specific gene targets across samples or sites. In addition, metagenomics/metatranscriptomics, give a non-targeted characterisation of the resistome of a sample. However, qPCR and metagenomics are often used less as they are comparably more expensive than culturing and PCR approaches. This is also the case for digital PCR (dPCR).

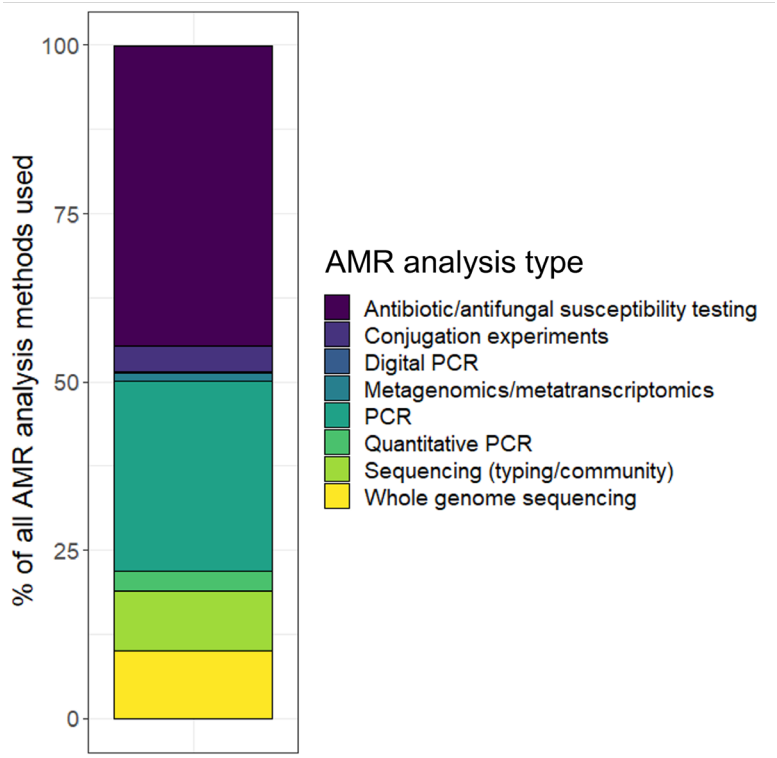

Figure 16 Proportion of different AMR analyses methods performed in studies found in the literature. Proportion shown as total percentage of all analyses occurrences (total number of analyses occurrences = 872).

3.2.3.3 Target microorganisms and genes

The microorganisms most targeted throughout the literature will reflect the sample type chosen and the research question. For example, enteric bacteria were isolated from faeces, to identify common gut pathogens. Unsurprisingly, the most targeted microorganism in culture was *E. coli/*other *Escherichia* species, with a total of 193 occurrences in the database (33%) (Figure 17a). This was followed by other Enterobacterales, such as *Enterobacter spp.* and *Salmonella spp.*, and other unspecified enteric bacteria in the order (Figure 17a). Other specific targets usually included human pathogens, such as *Campylobacter spp.*, *Pseudomonas spp.* and *Staphylococcus spp* (Figure 17a). Many of these genera contain zoonotic disease-causing species, that have been shown to spread easily through different environments, from animals to humans, and to also carry AMR (e.g., resistant *Campylobacter jejuni* (Marotta et al., 2019)), making them more likely to be target of interest. Notably, even though some fungi are also very serious human pathogens that exist in the environment, fungi were much less represented in the literature than bacteria. Of the fungi targeted, *Candida spp.* were the most common (Figure 17a).

It was much more likely for culture-based research to focus on specific bacteria, than all culturable bacteria (which are still only a small portion of all bacteria present), which leads to taxonomic bias in the literature. This finding was also highlighted by Torres et al., (2020) in their bibliometric analysis.

Over 25% of all resistance genes targeted in the literature were those conferring resistance to beta-lactam antibiotics (609 occurrences in the database), of which 93% were beta-lactamases (*bla* genes) (Figure 17b). The second most targeted resistance genes conferred resistance to tetracycline antibiotics (397 occurrences), and third to aminoglycosides (336 occurrences) (Figure 17b). When focusing on specific gene targets, the top five most targeted were *blaTEM* (91 occurrences), *tetA* (76 occurrences), *blaSHV* (72 occurrences), *tetB* (65 occurrences), and *sul1* (57 occurrences). However, if all variations of *blaCTX-M* were combined, this would be the most targeted by far, with 121 occurrences in the literature database, which is understandable as *blaCTX-M* variants are the most abundant of the ESBL genes and are highly clinically relevant (McNulty et al., 2014; Reuland et al., 2016).

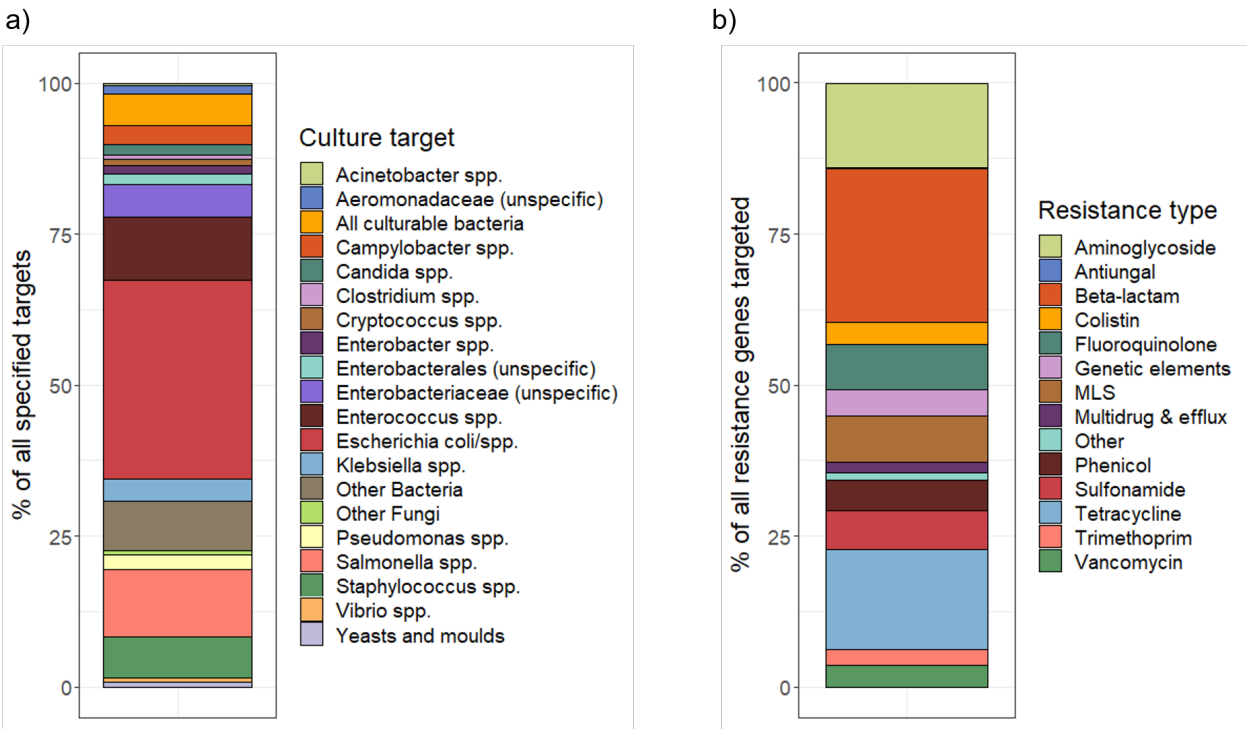

Figure 17 Proportion of different a) culture and b) resistant gene targets in studies found in the literature. Proportion shown as total percentage of all target occurrences (total number of culture target occurrences = 586; total number of resistant gene occurences = 2390). MLS = Macrolide, lincosamine and streptogramin.

3.2.4 Rationale for surveillance

The rationale for the studies included in the literature database was also recorded, although to some extent, this is subjective and at times unclear. If the rationale was unclear or did not fit into the main categories presented here, the rationale of the publication was recorded as “Other”. The most common rationale for carrying out research on AMR in wildlife was to investigate the dissemination of AMR throughout the environment (Figure 18). This was followed by, and at times overlapped with, assessing the spillover of AMR and resistance driving chemicals to the environment from anthropogenic contact (Figure 18). The rationales were less focused on explicit links to human health or testing new methods. Notably, some publications testing new methods were investigating the use of certain organisms as proxy species for measuring AMR in the environment (e.g., fish (Ballash et al., 2022) or bivalve molluscs (Grevskott et al., 2017) as indicators of AMR in the environment).

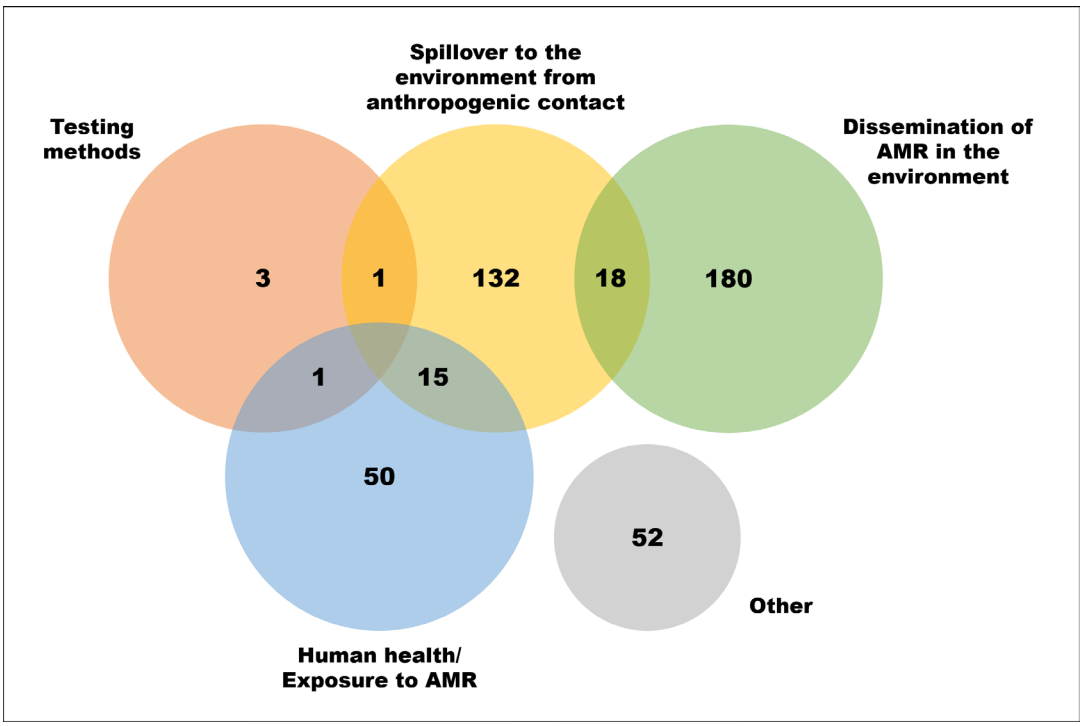

Figure 18 Number of publications for each rationale group.

3.2.5 Review limitations

This literature review was systematic within the bounds of the WoS database and was supplemented with publications from Google Scholar in a non-systematic way. Therefore, there may be research that has been excluded from this searching strategy, and thus could relate to the topic of AMR in wild animals and plants but may not be within the database produced here. The review was also constrained by only including articles published or translated in English, due to constraints in time and linguistic expertise. However, the quantity of academic journals included in WoS suggest that the results of this search are robust and representative of the global literature. Finally, biases may exist due to repetition in the same samples published although looking at different genes/resistance endpoints (as mentioned above), leading to bias and potential overrepresentation of certain countries, publication years, host taxa, and environment types. However, although analysis of the same samples may generate different publications, these will add to the depth of knowledge of different analytical targets, improving our overall knowledgebase.

3.2.6 Systems maps

We did not extract evidence of linkages between different environmental compartments (e.g., humans and wildlife, or wastewater and wildlife) in the literature review due to time constraints. However, based on reading the wider literature, as well as our expert opinion, we created the AMR systems maps for wild fauna (Figure 19) and wild flora (Figure 20) to

illustrate potential linkages between reservoirs of AMR, specifically highlighting the linkages between AMR reservoirs/drivers and wildlife that are likely to be the most important in driving AMR in wildlife populations. Many of these linkages lack robust evidence for their existence, especially across multiple systems/environments, and particularly in England. Because of that, these systems maps can be seen to highlight possible research priorities in understanding AMR in wildlife and the major anthropogenic and ecological drivers that influence it.

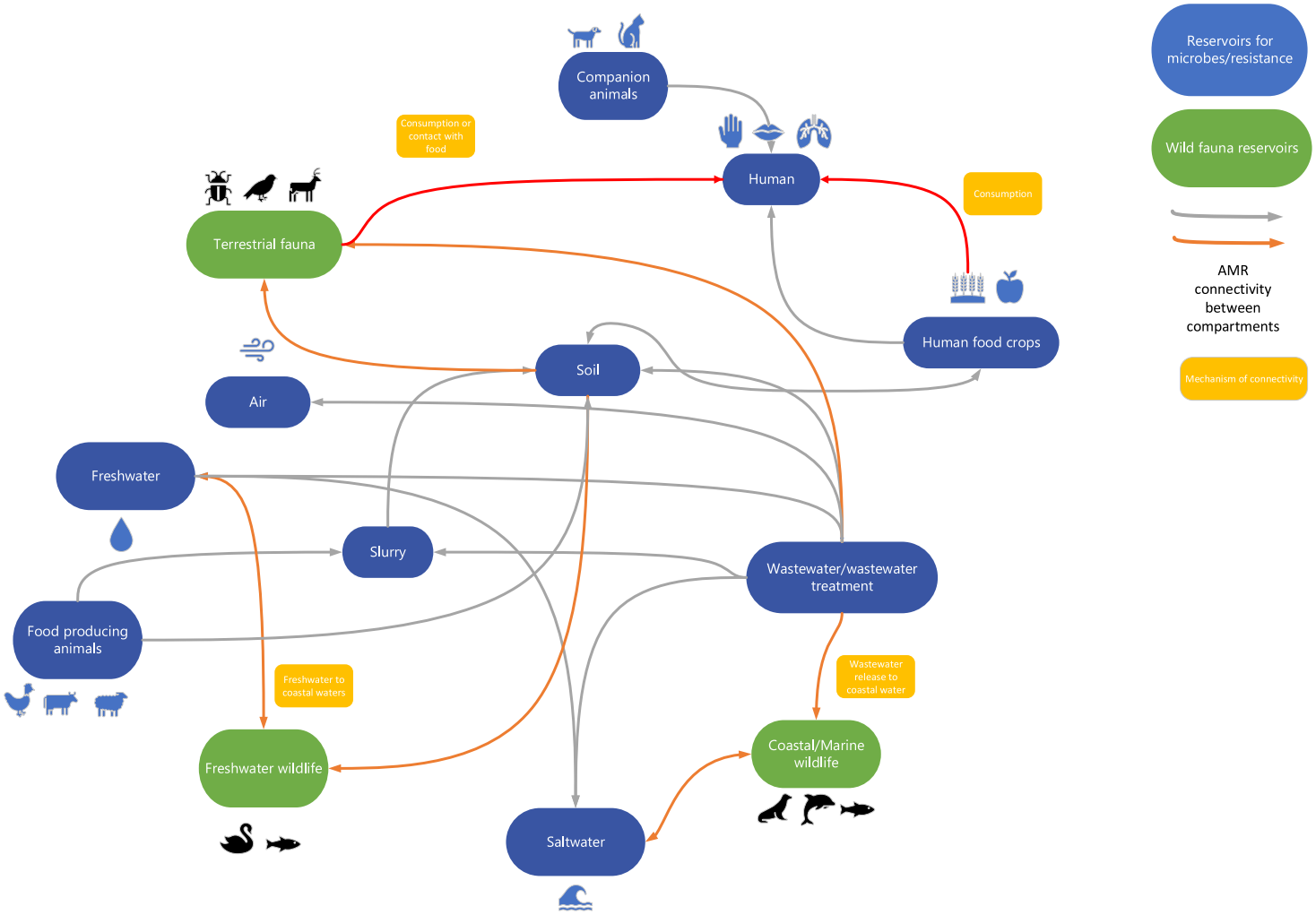

Figure 19 Systems diagram for AMR pathways relating to wild animal hosts.

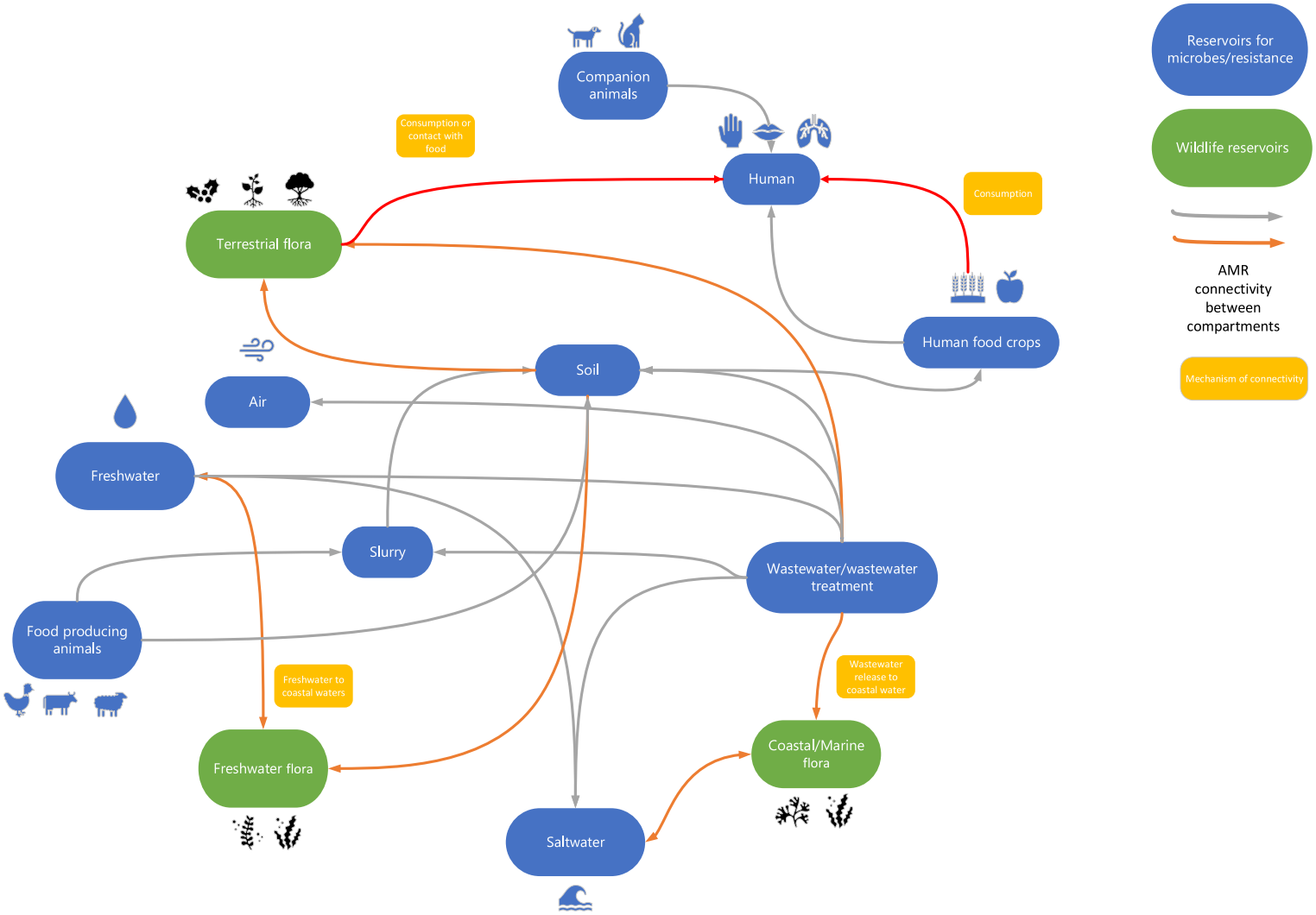

Figure 20 Systems diagram for AMR pathways relating to wild plant hosts.

4 Knowledge gaps

Whilst an integrated, One Health approach is increasingly being taken for the study of AMR, studies explicitly identifying the prevalence and composition of AMR in wildlife are still relatively few, especially in England. In the sections below, we have identified the key knowledge gaps in AMR and wildlife, both in the wider literature (Section 4.1) and in English wildlife surveillance (Section 4.2).

4.1 Research gaps identified in the literature

4.1.1 Invertebrates/Amphibians/Reptiles/Fish

Throughout the published literature on AMR and wildlife, amphibians, fish, invertebrates, and reptiles were all less studied than mammals and birds (Figure 9), with mammals and bird studies having almost five times as many publications targeted to them than all other organism types combined. Although some species of amphibians, fish, invertebrates, and reptiles are widespread and abundant in the environment and may cohabit with human populations, thus being important transmission routes in human disease (e.g., flies), their utility as a surveillance target may be hindered by difficulties in obtaining samples and sample processing. For example, amphibians and reptiles may both be rarer, and harder to capture than mammals or birds, making sampling more challenging. It may also be harder to recover faeces from fish, invertebrates, amphibians, and reptiles, which is a very commonly analysed sample type from mammals and birds. Difficulties with dissection of the animal and/or collection of faeces can be solved by destructive sampling, which is often done with smaller samples (e.g., homogenisation of whole insects). However, destructive sampling can be less desirable for ethical reasons than catch-and-release sampling or opportunistic faecal sampling, two processes often done with mammals and birds. The volume of sample may also be lower in these organism types, making extraction of DNA and detection of microbial/genetic targets of interest much harder. Alternatively, the overrepresentation of mammals and birds in the database may be a function of previous research priorities and sampling infrastructure, i.e., if many publications and existing surveillance efforts already focus on birds and mammals, new authors may be more likely to follow suit.

4.1.2 Plants

The lack of plant-based studies identified in the literature could be due to the perceived lack of importance of wild plant AMR carriage to human health. Unlike animals, plants rarely move and are less genetically related to humans, therefore may be deemed less likely to transmit human-related pathogens. For these reasons their potential utility as an indicator of the prevalence of AMR in the environment may be lesser than the potential utility of fauna. When searching for wild plant-based AMR publications, research on AMR in crop plants arose. Although these were not included in the database, it was evident that crop-based

studies far outweighed those on wild plants. This was likely due to them focusing largely on resistance to crop treatment, which has obvious and important food security implications. In addition, crop plants are grown to be consumed by humans, whereas wild plants may or may not be foraged, depending on whether the plant can be consumed safely and local human behaviour. Therefore, AMR present on crop plants (particularly those that are uncooked, that may harbour environmental bacteria carrying AMR) will be exposed to humans through consumption and could result in a human health outcome (e.g., colonisation or infection) (Stanton et al., 2022b).

4.1.3 Antifungal resistance

The lack of fungi-based studies in relation to AMR in wildlife is likely because fungal resistance is often only seen as a problem of crop pathogens and human pathogens, with few studies investigating environmental niches or deeming them important. Additionally, *Candida spp.* and *Aspergillus spp.* are often investigated in companion animals, yet rarely in wild animal species. Of the studies looking at wildlife, some have focused on low body temperature animals for wildlife health (e.g., *Batrachochytrium dendrobatidis* infection in amphibians (Lips, 2016)), which are of less relevance to humans and human health. Even in studies looking at fungal pathogens in the environment for human health reasons, many do not look for resistance; it is much more common to only look for fungal infections (e.g., Dutch elm disease). This lack of focus on fungal resistance also mirrors that of wider AMR research and programmes in general (Fisher et al., 2022), as fungi tend to be one of the lesser-studied human health threats (Rodrigues & Nosanchuk, 2020). For example, Cryptococcal meningitis receives less than a quarter of the research funding than that of bacterial *Neisseria meningitidis* yet is responsible for 20 times more deaths (Rodrigues & Albuquerque, 2018). This is concerning, as fungal pathogens such as *Cryptococcus neoformans*, *Candida spp.* and *Aspergillus fumigatus* can be highly resistant and have high mortality rates (WHO, 2022), and have been widely isolated from natural environmental niches (i.e., non-wildlife niches) (e.g., azole-resistant *A. fumigatus* (Fraaije et al., 2020)). In summary, this lack of focus on antifungal resistance in wildlife leaves us with very little understanding of the potential reservoirs of clinically relevant resistant pathogenic fungi in the environment.

4.2 UK wildlife surveillance knowledge gaps

4.2.1 Purpose-designed wildlife AMR surveillance schemes

We were unable to identify any wildlife surveillance schemes that had the surveillance of AMR in wildlife as their core focus. As a result, there is a lack of available information describing trends in AMR carriage across wildlife species in England, as well as data on trends in AMR over time and space. As a result, it is not currently possible to answer with any reliability what role wildlife species in England play as reservoirs and transmitters of AMR. We identified three schemes that do currently collect AMR data whilst performing pathogen focussed research, including the Diseases of Wildlife scheme (APHA), the garden Wildlife Disease scheme (IOZ), and to a lesser extent, the Cardiff University Otter

surveillance scheme that has performed some pilot work in this area. All these schemes are passive, receiving either diseased animals, or those that are killed via road traffic. Whilst this approach has obvious advantages in terms of cost and is effectively able to perform disease surveillance and scanning in wildlife, as discussed in section 2.2, passive approaches also have important limitations. AMR data from these schemes are currently not able to answer key questions regarding the prevalence of AMR in non-diseased wildlife populations, or the role of anthropogenic activities in driving AMR in wildlife. These important questions are best addressed by active surveillance schemes, targeting sampling across spatial or anthropogenic gradients.

4.2.2 Aquatic organisms

Whilst schemes that target aquatic organisms appeared to be well represented (seven of the 13 schemes included a freshwater component), only two schemes target fully aquatic organisms (the Environment Agency's Fish disease surveillance and the UKCEH Fish tissue archive), and one targets a semi-aquatic mammal (the Cardiff University Otter Surveillance scheme). This is important, as there is increasing evidence that the aquatic pathway is a significant, and high profile, route of AMR and resistance driving chemical contamination, particularly via wastewater and agricultural activities (Environment Agency, 2022; Neher et al., 2020). Aquatic environments are also subject to high levels of temporal variation in water chemistry and biological contaminants, driven by hydrological events (Rode et al., 2016). Because of this, monitoring AMR in species that act as "environmental integrators" may be an important approach to understanding how anthropogenic activity drives the dissemination of AMR to wildlife, and how wildlife can act as indicators of this activity.

5 Recommendations

When making recommendations for a sampling approach that utilises wildlife to better understand the environmental dimensions of AMR, several factors need to be taken into consideration, as described below.

5.1 Methodological recommendations

5.1.1 Consideration of host wildlife characteristics

Wild flora and fauna have distinct behavioural and ecological characteristics that influence their usefulness for understanding AMR in wildlife. Ecological characteristics such as habitat specificity and distribution influence the probability of wildlife exposure to AMR (in the form of resistant microorganisms and antimicrobial resistance driving chemicals). The importance of host characteristics highlights the need to consider these variables in surveillance planning, and tailor them to the research question. For example, targeting species that are restricted to pristine environments may be useful for understanding the role of wildlife microbiomes as reservoirs of AMR mechanisms, but would not be useful if the intention is to understand how anthropogenically-impacted environments affect levels of AMR in wildlife. The trophic behaviour of organisms is also an important characteristic. Apex predators such as predatory birds and mammals may represent an important host for AMR due to their close association with the microbiomes of the organisms they prey upon, similar to bioaccumulation that occurs for some persistent chemicals through trophic levels (Ali & Khan, 2019). However, this theory is largely untested, and conflicting information exists (Vittecoq et al., 2016). Where comparisons have been made, the general trend is that carnivorous and omnivorous species are generally the most at risk of carrying AMR, highlighting that trophic level is potentially an important characteristic (Vittecoq et al., 2016). Another example of important characteristics to consider can be seen in aquatic organisms that are filter feeders, due to their high level of contact with the surrounding environment, and the degree of overlap the species has with human habitation and human activity.

5.1.2 Understanding linkages with AMR reservoirs

Whilst not quantified in our review of the AMR in wildlife literature, we observed that studies that explicitly linked AMR in wildlife with other reservoirs such as human, domestic animal or other environmental compartments, were lacking. This is important, as to achieve One Health aims, it is necessary for studies to investigate the dissemination of AMR and relative weighting of reservoirs throughout different One Health compartments (human, animal, environment). As a result, we recommend that any surveillance of AMR in wildlife, where possible, be linked or performed in combination with measurements of AMR from likely sources, such as human and domestic animal populations, wastewater emissions to aquatic environments, or sewage sludge applications to land. Additionally, co-located chemical measurements that encompass known antimicrobial resistance driving chemicals such as antibiotics, fungicides and metals are desirable to help interpret AMR data from wildlife.

5.1.3 Standardised AMR analysis approaches

AMR can be measured using a wide variety of techniques, including culture-based and molecular methods (Anjum et al., 2017). The choice of method has a large influence on what aspect of AMR is examined. Culture-based methods have the advantage of high degrees of selectivity, allowing pathogenic groups of microbes to be isolated and examined in more detail, with the added advantage of a high degree of comparability with clinical microbiology data. Molecular methods are highly versatile, enabling the analysis of specific AMR genes and characterisation of the whole resistome directly on environmental samples. A challenge often faced by researchers is trying to compile environmental resistance data when there is a lack of consistency in monitoring methods (Allen et al. 2010) and therefore, a lack of consistency in research and policy outcomes (Eckert et al. 2016). Thus, to allow comparability across studies, method standardisation is needed. International initiatives have highlighted recommended approaches (Pruden et al., 2021; Ligouri et al., 2022), and we recommend that any new monitoring schemes in the UK attempt to use widely recognised approaches where possible. Based on these recommendations, we suggest the following core surveillance approaches:

A. Culture-based analysis of extended spectrum β-lactamase (ESBL)-producing *Escherichia coli*. This enables quantification and characterisation of a common enteric and bloodstream pathogen that is widespread in both human, animal and environmental compartments. The proportion of resistant colonies can be used as an indicator of AMR prevalence within a given environment.
B. Quantitative PCR (qPCR) of key resistance genes. qPCR is a highly sensitive method that can be used to quantify the prevalence of resistance genes across a wide range of sample matrices (water, soils, sediments, and faecal samples). It has been proposed that key genes such as *intI1* (the class 1 integron-integrase gene, that is often used as a marker for pollution and AMR (Gillings et al. 2015), *sul1* (sulfonamide resistance), *bla*CTX-M (a family of class A beta-lactamase genes that confer resistance to cephalosporins), and *vanA* (vancomycin and teicoplanin resistance) should be used as a core set of resistance and resistance associated genes to monitor in aquatic environments (Berendonk et al., 2015; Ligouri et al., 2022).
C. Metagenomic sequencing, based on high throughput DNA sequencing. Metagenomic sequencing has the advantage of being non-selective, in that no prior knowledge is needed about the sample before producing an AMR profile. As a result, metagenomic approaches can profile a wide array of resistance genes simultaneously, without the need for *a priori* target choice. Metagenomic data can also be re-analysed, allowing newly identified resistance genes to be retrospectively found in datasets.

5.1.4 Archiving of samples and data

Whilst standardisation of analytical methods should be attempted, a major limitation is that the field of genomic analyses is constantly evolving, particularly when it comes to high throughput DNA sequencing (HTS) and the subsequent bioinformatics analysis of HTS data. New data generation methods, and variations in the approaches used to analyse such data can have a profound influence on AMR data interpretation. Whilst this is unavoidable, open

approaches to archiving samples (and/or extracted DNA) and raw data, can encourage the re-analysis of data, improve reproducibility and allow the latest developments in wet-lab and data science to be applied to AMR surveillance schemes. As such, we strongly encourage consideration of how samples (including extracted nucleic acids) and data are stored when designing a surveillance scheme, with a view to potentially reanalysing in the future.

5.2 Recommendations for AMR surveillance in wildlife in England

There are several reasons for performing AMR surveillance on wildlife, including for public health and as a tool to understand disease ecology and environmental pollution (see Section 2.2). In this section we focus our recommendations on one specific aspect of AMR and wildlife; the use of wildlife as “integrators” of AMR to better understand AMR in the environment. This is analogous to the use of wildlife as sentinels of other forms of pollution, particularly persistent chemicals that have the potential to bioaccumulate. Indeed, four of the schemes we evaluated (Predatory Bird Monitoring Scheme, the National Fish Tissue Archive, the Cardiff University Otter Surveillance scheme, and the National Honey Monitoring Scheme) either have chemical surveillance as their main or secondary purpose. Our recommendations are also based on practicality. Setting up entirely new, purpose designed monitoring schemes across multiple species and trophic levels may be ideal in terms of structure and statistical robustness, but not practical in terms of resources. These recommendations are also influenced by the systems maps in Section 3.2.6 that highlight the potential linkages between the sources of AMR and resistance driving chemicals and AMR in wildlife.

5.2.1 Aquatic (freshwater) wildlife surveillance

Aquatic environments are subject to well-characterised sources of ARGs, AMR microorganisms and AMR driving chemicals, in the form of treated and untreated wastewater, as well as land use runoff (e.g., from livestock and crop agriculture, landfill, and aquaculture) (see systems map, Figure 19). As a result, coastal and freshwater habitats are a high priority for monitoring AMR in the environment (Environment Agency, 2022).

Fish are an obvious wildlife target for aquatic AMR surveillance, due to their presence across a wide variety of freshwater habitats and their position in the food chain. Perch-like (Perciformes) and carp-like (Cypriniformes) fish were the most studied fish type in the literature (Section 3.2.2, Figure 12), although the relatively low representation of wild fish in AMR studies (12/453 publications) mean that these figures are likely prone to bias by a limited studies and research groups. When selecting target species, it is important to choose species that are abundant and widely distributed. Another important linked characteristic is species that are found across both impacted and non-impacted environments, to enable comparisons of spillover across anthropogenic gradients. The Environment Agency have a well-established capability in fish surveillance in England through their fish population surveillance activities (not evaluated in this report) and their fish disease surveillance activities. As a result, we recommend utilising this existing surveillance activity to target

species such as roach (*Rutillus rutillus*) which are found across a range of freshwater habitats across England. An important research need is to understand what type of sample to collect. Faecal or intestinal samples were the most common sample type for wild fish AMR studies (e.g., Ballash et al., 2022), and may best represent the integration of feeding and habitat choice behaviour. However, gut and faecal samples likely require destructive sampling, which is not generally desirable for a national surveillance scheme, especially for vertebrate species. As a result, we recommend trialling in-field mucus swab samples, which are non-destructive and represent the interface between the fish microbiome and the environment they are exposed to, as well as destructive intestinal sampling.

Waterfowl were one of the most targeted bird groups, with the order Anseriformes (ducks, geese, and swans) comprising 7% of the 1,146 instances within wild bird-AMR studies (Figure 11). There are several features that make wildfowl a good target for AMR surveillance. Firstly, wildfowl are well-characterised carriers of zoonotic disease-causing organisms, including bacterial pathogens such as *Campylobacter* (Wysok et al., 2022) and *Escherichia* (Ewers et al., 2009), as well as avian influenza. They are abundant and widespread, found in the majority of waterbodies in England, including in water contaminated with sewage and in highly agricultural catchments.

Our final suggestion for freshwater monitoring is to incorporate monitoring from an apex predator such as the Eurasian Otter. Throughout the literature, those in the Mustelid family, which includes otters, were the most targeted host taxa within the Carnivora order. There is a well-established (since 1992) Otter surveillance programme run by Cardiff University that collects otters found dead in England, Scotland, and Wales for post-mortem examination. Research into persistent chemicals is carried out on these carcasses (e.g., Kean et al., 2021; O'Rourke et al., 2022), providing high quality contextual data for AMR research. Pilot work has been carried out on AMR, including culture-based analysis of *E. coli* and metagenomics, and there are archived faecal-rectal samples dating back ~20 years, making this a rich resource for generating baseline AMR data. One possible disadvantage is the fact that this is a passive surveillance scheme, meaning that sampled otter carcases may not be representative of the wider population. In addition, changes in AMR prevalence may occur post-death in the time taken to find and submit otter carcasses. Otters also represent a semi-aquatic lifestyle, and there may be important terrestrial influences on their AMR burden. Despite this, we believe these disadvantages are outweighed by the potential advantages of studying AMR in an important and widespread apex predator.

5.2.2 Aquatic (coastal and marine) wildlife surveillance

As highlighted in the fauna systems map in Figure 19, the main likely drivers of AMR contamination in coastal waters are microbiological and chemical pollution from rivers and both treated and untreated wastewater.

Gulls (and their relatives in the suborder Lari) were popular target host taxa found in coastal areas, comprising 9% of the 1,146 instances within wild bird-AMR studies (Figure 11). However, many species of gull have a high association with human habitation and waste, with some species being found in urban areas, at refuse disposal sites and as common

visitors to wastewater treatment plants (Coulson, 2015). The high level of mobility mean that it may be a challenge to link patterns of AMR with a particular driver, which may result in gulls (and their relatives) being unsuitable for surveillance of AMR.

Our recommendation is to perform pilot work to ascertain levels of AMR in common coastal marine fish or invertebrate species. Common filter feeding bivalves such as the blue or common mussel (*Mytilus edulis*) are widespread, both in the wild and as a farmed food source. Although not covered in our literature as we did not include farmed animals, studies of AMR in farmed shellfish (and other farmed seafood) are more common, as the high levels of antibiotic usage in some sectors of the farmed seafood industry (e.g., salmon) have led to concern about AMR generation and transfer to humans. Wild filter feeding species such as mussels have a high degree of contact with the surrounding environment due to their filter feeding habit, and these organisms are well known to host clinical pathogens that are derived from contamination of coastal areas.

5.2.3 Terrestrial wildlife surveillance

There are a wide range of potential terrestrial targets for terrestrial AMR monitoring, reflected by the fact that this was the most sampled habitat within the research literature (Figure 8). Our system map (Figure 19) identified direct interactions with wastewater treatment works, agricultural applications of sewage sludge and slurry to soil, and agricultural applications of biocides (both to flora and fauna) as the main possible routes of AMR dissemination into terrestrial biomes. Because of that, our recommendations are focused on species that may inform on the relative impact of these activities in disseminating AMR in terrestrial wildlife species. Although terrestrial wildlife surveys were well represented in our evaluation, few of these fully met the criteria to recommend building on these activities for AMR surveillance with the intention of using wildlife as integrators or indicators of AMR in the environment. The best developed schemes in terms of integrated wildlife disease surveillance (the Diseases of Wildlife scheme and the Garden Wildlife Disease scheme) are both passive schemes, for which it would be challenging to link anthropogenic activities with AMR burden. This is particularly true for schemes that examine AMR in birds, for which high levels of mobility make inferring these links more challenging.

One key terrestrial group, and one of the most frequently studied mammal orders for AMR, is Rodentia, which are small rodents such as mice and rats. In the UK, these small rodents are both abundant and widespread, and are found in both natural and highly modified environments. Rodents have been shown to host a wide variety of zoonotic diseases (Morand et al., 2015) and some species such as rats are renowned for the societal implications of their disease carrying properties. Both live and fatal trapping of rodents is carried out as a part of routine pest control and research into rodent populations, thus may provide opportunities to generate baseline data on AMR prevalence in populations across gradients. Although there is a lack of existing schemes to build such an activity on, we recommend that pilot work is conducted to ascertain the usefulness and feasibility of small mammal surveillance for AMR.

Understanding the potential role of soil applications of sewage sludge and manures in disseminating AMR to soils (and potentially surrounding freshwater environments) is an important topic. A possible target group to examine as integrators of soil pathways are earthworms (suborder *Lumbricina*). Common species such as *Lumbricus rubellus* are found across a wide range of habitats, including agricultural soils, and are closely associated with soil contaminants due to their geophagous lifestyle. Previous laboratory- or farmed-based research has highlighted that earthworm guts host a wide variety of antimicrobial resistance genes, and that this can be influenced by the application of livestock manure (Tian et al., 2021; Zhou et al., 2020), sewage sludge (Cui et al., 2019), and metals and antibiotics (Wang et al., 2019).

5.2.4 Wild flora as a target for AMR surveillance

Research into wild flora as a host of resistant organisms were in the minority in the wider literature, with only five studies identified. This is likely be due to the perceived lack of importance of AMR in wild plants to human and domestic animal health. This contrasts with the perceived importance of AMR on crop plants, which may expose human populations to AMR (e.g., through food consumption and occupational hazards). For these reasons their potential utility as an indicator of the prevalence of AMR in the environment may be less than fauna. As a result, we currently do not recommend any surveillance based on wild flora unless further evidence to their importance in the environmental dimension of AMR emerges.

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