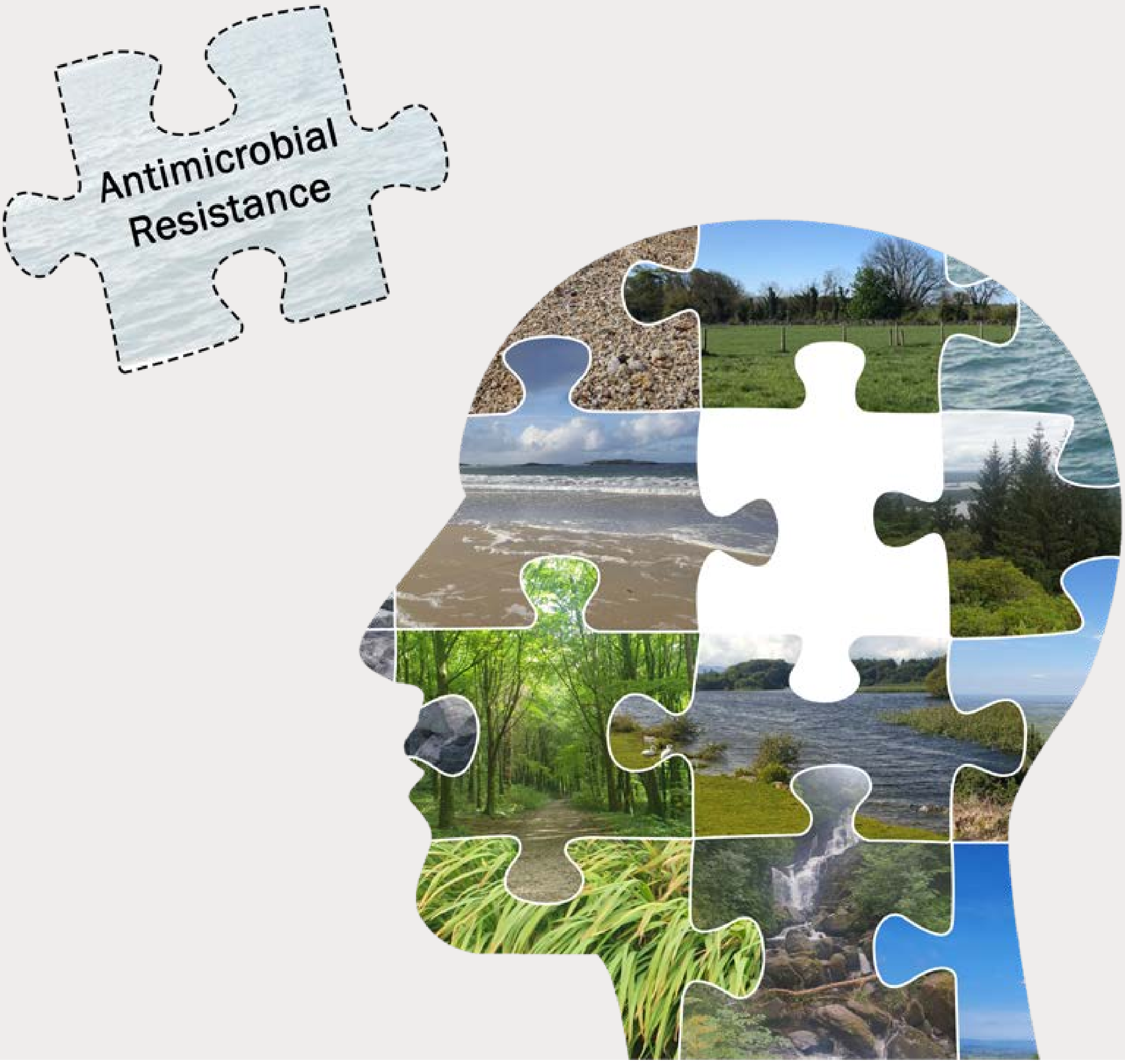
Gap analysis of research needs to understand the environmental dimension of antimicrobial resistance in preparation for Ireland's second One Health Action Plan on Antimicrobial Resistance 2021-2025 (iNAP2)



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Gap analysis of research needs to understand the environmental dimension of antimicrobial resistance in preparation for *i*NAP2

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DISCLAIMER

This report is based on a review of published material from 2015 to April 2021. More recent data may have become available since this review was completed.

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Executive Summary

Antimicrobial resistance (AMR) is one of the greatest global threats we are facing in today's world. The role of the environment in the emergence and dissemination of AMR and the impact it may have on human and animal health is an area of increasing focus. As recognised in Ireland's first National Action Plan on Antimicrobial Resistance (*i*NAP), adopting a One Health approach is key to effectively tackling the problem of AMR. To date, the environmental dimension of AMR has been the part of the One Health paradigm that has not received enough focus. Developing a better understanding of the role of the environment in the emergence, dissemination, transmission and persistence of AMR is crucial to inform future strategies and policy developments, including the second iteration of *i*NAP.

The aims of this research gap analysis were to:

1. Complete a comprehensive review of previous and ongoing research in the area of antimicrobial resistance and the environment in Ireland from 2015 – 2021.
2. Identify key knowledge gaps in the environmental dimension of AMR in Ireland to inform the second iteration of *i*NAP through evaluation of the findings of a survey and stakeholder workshop relating to AMR and the environment.
3. Prioritise the research needs identified.

A comprehensive analysis of previous and ongoing research relating to the occurrence, risk, fate and transport of antimicrobial residues and AMR to and within the environment was completed. The focus of this analysis was on national considerations in preparation of the work programme for the second iteration of *i*NAP. Consideration was also given to emerging issues identified internationally and how these may relate to the Irish situation. Only literature on research relating to AMR and the natural environment carried out in the Republic of Ireland published from January 2015 to April 2021 was included. However, the authors acknowledge that research has been carried out in this area prior to 2015. Research into the environmental dimension of AMR in Ireland to date has concentrated primarily on the role of human waste as a source of AMR and the potential for subsequent transmission via the environment to humans. Findings to date demonstrate that multi-drug resistant organisms including those on the WHO's list of priority pathogens are present in the Irish aquatic environment with human and animal waste being identified as most likely sources. There is a need

to better understand the consequences of releasing such waste via wastewater discharges and land spreading of biosolids on all environmental compartments.
To gain an insight into the key knowledge gaps in the environmental dimension of AMR from an Irish perspective a survey was developed and circulated to 78 researchers who have previously worked, or are currently working, in the area of AMR in human, animal and environmental sectors in Ireland. The response rate was relatively low with 22 responses received, likely reflecting the limited number of researchers currently active in the AMR and environment field in Ireland. Findings of the survey and a poll circulated at the joint EPA/HSE/ESRI 'Environment, Health and Wellbeing' virtual conference held on November 26th 2020 informed the topics for discussion at the AMR and the Environment stakeholder workshop held on April 21st 2021. The workshop included representatives from research institutions, funding organisations and government bodies.

Although a wide range of views were expressed by respondents to the poll and survey, and participants in the stakeholder workshop, a number of common themes were identified in relation to key knowledge gaps and future research needs. These have been ranked as high, medium and low priorities based on current knowledge available in Ireland. Development of appropriate policies, risk management strategies and mitigation measures rely on the generation of appropriate evidence. Investment in research in the priority areas identified will support implementation of Ireland's National Action Plan on Antimicrobial Resistance and demonstrate Ireland's commitment to tackling the global challenge of AMR.

1. Introduction

Antimicrobial Resistance (AMR) is recognised as one of the greatest threats we are facing in today's world. The emergence and spread of AMR as a result of both appropriate and inappropriate antimicrobial use (AMU), as well as inadequate infection prevention and control measures is placing a huge burden on both health services and economies worldwide. In 2019, it was estimated that AMR resulted in extra healthcare costs of approx. €1.1 billion per annum in the European Union (EU)/ European Economic Area (EEA) (OECD and ECDC, 2019). There is some evidence to suggest that the current COVID-19 pandemic may amplify the problem of AMR due to the inappropriate use of antimicrobials, as it is estimated that up to 60% of those infected with COVID-19 receive antimicrobials unnecessarily (WHO, 2020).

Approximately 33,000 deaths were caused by antibiotic resistant bacteria in the EU/EEA, in 2015 alone (Cassini *et al.*, 2019). Across the globe, the number of deaths attributable to AMR was estimated at 700,000 per annum in 2014 and is expected to reach 10 million per annum by 2050, if corrective and preventative action is not taken now (O'Neill, 2014).

Several initiatives have been put in place in recent years to tackle the ongoing and escalating problem of AMR across the globe. This includes the establishment of the global action plan against AMR by the World Health Assembly in 2015 (WHO, 2015), as well as the development of the EU One Health Action Plan against AMR by the European Commission in June 2017 (European Commission, 2017). Although no country can tackle the global problem of AMR alone, the development of AMR action plans by individual countries is recognition globally of the need for a cohesive approach to address the problem of AMR at national level. In response to this, Ireland's first '*National Action Plan on Antimicrobial Resistance'* (*i*NAP) (2017-2020) was published in October 2017 (DOH, 2017). The importance of recognising the interconnection between human, animal and environmental health and taking a 'One Health' approach to tackle the problem of AMR is highlighted by the global, European and national action plans, including *i*NAP.

The role of the environment in the emergence and spread of AMR and the impact it may have on human and animal health is increasingly recognised as an area requiring more focus (WHO, 2015; DOH, 2017; European Commission, 2017). The need for further research to better understand the role of the environment in the emergence, dissemination, persistence and transmission of AMR is crucial to inform future strategies and policy developments, as highlighted in both the European and Irish actions plans on AMR (DOH, 2017; European Commission, 2017).

The publication of *i*NAP in 2017 outlined five key objectives to tackle the problem of AMR in Ireland, in human, animal and environmental health sectors (DOH, 2017). In relation to the environment, interventions and activities considered a priority in the prevention, surveillance and fight against AMR are outlined under *i*NAP's objective 1) raising awareness and knowledge around AMR and antimicrobials, 2) enhancing AMU and AMR surveillance, and 3) reducing the spread of AMR (DOH, 2017). Following a visit to Ireland in October 2019 by the European Centre for Disease Prevention and Control (ECDC) and the European Commission's Directorate General for Health and Food Safety to discuss policies relating to AMR, a report highlighting progress made in Ireland in the fight against AMR was published (ECDC, 2019).

As outlined under *i*NAP's objectives 1 and 3, interventions relating to the improvement of hazardous waste management and treatment, and education around the appropriate disposal of antimicrobials were to be carried out in order to minimise the spread of and enhance knowledge of antimicrobial residues in the environment (DOH, 2017). In line with these objectives, work on improving water quality, water and wastewater treatment processes and facilities has been carried out. Efforts to raise awareness around the appropriate disposal of antimicrobials in Ireland have also begun (ECDC, 2019). With the implementation of the 'National Hazardous Waste Management Plan' (NHWMP) (2014 – 2020) progress has been made in improving management of hazardous waste with the aim of reducing harmful wastes, including antimicrobials, from entering the environment (ECDC, 2019). In addition to this, a One Health disposal group has been established and is reviewing potential options for a national system of collection for medicines used in both human and animal sectors. The next iteration of the NHWMP (2021-2027) aims to build on progress already made and continue working to improve the management of hazardous wastes in Ireland, including human and veterinary medicines. The development of national systems for collection of human and veterinary medicines has been included in the recommendations outlined in the draft NHWMP plan (EPA, 2021).

Another one of *i*NAP's key goals was to improve AMU and AMR surveillance across human, animal and environmental divisions. Under objective 2, activities and interventions to be carried out in relation to the environment included research on areas such as the impact of AMR and antimicrobial residues in the environment, key hotspots, and methods for the detection and elimination of AMR and antimicrobials in and from the environment, as well as the monitoring and reporting of antimicrobials in specific environmental compartments in line with EU monitoring criteria (DOH, 2017).

Currently in Ireland and elsewhere there is no routine monitoring of the environment for the presence of antimicrobial resistant bacteria (ARB) or antimicrobial resistance genes (ARGs). Data available to date arises out of research projects. Details of ongoing projects relating to AMR in the environment in Ireland are available in Section 3.

Under the EU Water Framework Directive, surface water monitoring for pollutants is undertaken across all EU member states (EPA, 2021). Each member state is required to monitor surface waters for priority and watch list substances. Although at present there are no antimicrobials included in the list of priority substances (EPA, 2021), they are included in the watch list (European Commission, 2020). Surface water surveillance for the watch list substances is carried out in order to monitor potentially hazardous pollutants which may be later added to the priority list. In Ireland, surface water surveillance for watch list antimicrobials has been carried out since 2016 by the Environmental Protection Agency (EPA) (ECDC, 2019). When initially published in 2015, the watch list included three macrolide antibiotics (erythromycin, clarithromycin and azithromycin) (European Commission, 2015). Two more antibiotics, amoxicillin and ciprofloxacin, were later included following an update in 2018 (European Commission, 2018). From 2016 to 2019, monitoring was carried out at four surface water locations across Ireland. With the exception of erythromycin levels exceeding the required detection limit at two of the locations during one round of sampling in 2019, levels of all watch list antibiotics monitored for have remained below the detection limits since 2016 (European Environment Agency). In 2020, the watch list was updated again which resulted in the exclusion of the macrolide antibiotics; however, two more antibiotics were included, sulfamethoxazole and trimethoprim (European Commission, 2020).

In addition to monitoring surface waters for the priority and watch list substances, each EU member state must also identify and monitor river basin specific pollutants of national, regional or local concern (European Commission, n.d.). Although in Ireland this list does not contain any antimicrobials at present (European Communities Environmental Objectives (Surface Waters) Regulations, 2009), a review of the river basin specific pollutants list is currently being carried out by the National Aquatic Environmental Chemistry Group. This group chaired by the EPA contains members of various national agencies and was set up in 2018 with the aim to review hazardous chemicals being monitored in the aquatic environment in Ireland and to identify those of emerging concern (Ireland, Department of Housing, Planning and Local Government (2018).

In 2019, Ireland’s first ‘One Health’ report on AMU & AMR was published, however, this was limited to the inclusion of data on humans and animals (DOH, 2018). In a move towards adopting a one health approach, and in line with *i*NAP’s objective 2, Irelands next ‘One Health’ report on AMU and AMR will integrate human, animal and environmental data, by extending reporting of AMU and AMR surveillance in humans and animals to include data on AMR in the environment (DOH, 2017; ECDC, 2019).

As the three-year time frame (2017-2020) of *i*NAP has come to an end, a review of the current AMR situation in Ireland and activities completed relating to AMU and AMR surveillance and research in human, animal and environmental sectors in recent years is key in preparation of the second iteration of *i*NAP.

This report includes a comprehensive review of research performed and published in the area of AMR and the natural environment in the Republic of Ireland from January 2015 to April 2021 and describes a gap analysis completed to identify key knowledge gaps in this area and recommendations for research priorities. Where we refer to AMR, in line with the *i*NAP, we are primarily referring to resistance to antibiotics as research performed in Ireland on AMR from 2015 to date has predominantly focused on bacterial resistance to antibiotics.

2. Review of Recently Published Research in Ireland (2015-2021)

2.1 Antimicrobial Resistance in the Environment in Ireland

To assess the current situation and knowledge on AMR and the environment in the Republic of Ireland, a review of previous and ongoing research in this area was carried out. National and international funding organisations were contacted in order to gather information on both previous and ongoing AMR related research funded in Ireland. Following compilation of all AMR related projects, all principal investigator details were collected and a search of relevant databases for publications relating to the environment was completed. For the purposes of this review, only literature on research relating to AMR and the natural environment carried out in the Republic of Ireland published since 2015 was included. Research published prior to 2015 was not in scope for the current exercise.

The publications reviewed in Section 2 primarily focus on human waste and the aquatic environment. This reflects the research performed in Ireland and published throughout the time period (January 2015 – April 2021) relating to AMR and the natural environment in Ireland. There are a number of AMR related projects currently ongoing in Ireland on other aspects of the environment including soil, plants and the spread of AMR through animal manure and these are outlined in Section 3. As these projects are currently ongoing, we expect publications detailing findings of this work will be available in the coming years. There are also other research projects and publications from Irish researchers that do not contain samples from Irish environments.

The scope of this review and research gap analysis is limited to the environmental dimension of AMR. We acknowledge there are overlaps with the human and animal sectors, but publications limited to either or both sectors to the exclusion of the environment are not included. A number of the studies discussed throughout this review did not receive any specific funding from agencies in the public, commercial, or not-for-profit sectors for the research carried out in Ireland, however where financial support was received, this has been indicated throughout the review.

2.1.1 Potential sources of antimicrobial resistance in the Irish environment

Human and animal waste have been highlighted as major sources of AMR in the environment and have the potential to enter the environment via wastewater discharges, land spreading applications, agricultural runoff etc. (DOH, 2017). Despite animal waste being a potentially major source of AMR in the environment, there has been a lack of research published in this area in Ireland from 2015 to date. In relation to human waste, studies have been carried out in Ireland in recent years which have identified the presence of ARB and ARGs, including carbapenemase-producing *Enterobacterales* (CPE) in various wastewater sources, and are discussed throughout this Section 2.1.1. CPE have been listed as 'critical' in the World Health Organisations list of priority pathogens developed in 2017 (WHO, 2017) and are one of the most concerning types of ARB at present due to the serious risk they pose to human health and the lack of effective treatment options available in some cases (DOH, 2017). CPE were declared a public health emergency in Ireland in 2017 by the then Minister for Health, Simon Harris, to coincide with the publication of *i*NAP.

Mahon *et al.* (2017) reported the detection of CPE in a municipal sewage system including storage tank, collection system and outflow in 2017. The municipal sewage stream which has no known link to any healthcare facility, was contaminated with both NDM-producing *Escherichia coli* (*E.coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) and was investigated following detection of CPE in a nearby freshwater stream. Among isolates detected, resistance to β-lactams, quinolones, fluoroquinolones, aminoglycosides and tetracycline was noted (Mahon *et al.*, 2017). The detection of NDM-producing *Enterobacterales* prompted further investigation of the surrounding aquatic environment of which results are discussed in Section 2.1.2. In this particular study, the discharge of untreated wastewater was believed to be the probable source of CPE emergence and dissemination in the surrounding environment (Mahon *et al.*, 2017).

Similar to Mahon *et al.* (2017), NDM-producing *E.coli* was also detected in municipal wastewater by Cahill *et al.*, (2019) in 2017. In this particular study, hospital wastewater and wastewater collected downstream following entry of hospital wastewater into the municipal stream were also positive for CPE harbouring the carbapenemases KPC, OXA-48, VIM and IMP. All CPE recovered were multi-drug resistant, exhibiting resistance to β-lactams, quinolones, fluoroquinolones, aminoglycosides, phenicol, trimethoprim and tetracycline. Although CPE were present in all sample types and based on the overall findings, Cahill *et al.* (2019) reported hospital wastewater as a potentially significant contributor of

CPE in municipal wastewater, as a higher prevalence of CPE was detected in hospital wastewaters in comparison to municipal.

The EPA-funded project 'Hospital effluent: impact on the microbial environment and risk to human health' demonstrated the impact of hospital wastewater on municipal wastewater with regards to AMR (Morris *et al.*, 2015). Both hospital and municipal wastewaters contained high levels of AMR *E.coli* exhibiting resistance to a range of antimicrobials including penicillins, aminoglycosides, sulphonamides, fluoroquinolones, cephalosporins and tetracycline. Morris *et al.* (2015) indicated that hospital wastewaters were not the main driver of all AMR *E.coli* in municipal wastewaters and the environment, particularly with regards to resistance to ampicillin and streptomycin. However, as indicated by Morris *et al.* (2015), hospital wastewaters do appear to be a potentially major contributor of resistance to certain antimicrobials e.g. sulphonamides, fluoroquinolones and tetracycline in municipal wastewaters and the environment.

Overall, the findings by both Cahill *et al.* (2019) and Morris *et al.* (2015) highlight the need for the development and implementation of guidelines relating to the appropriate management and surveillance of not only hospital wastewaters but also municipal wastewaters and the receiving environments.

Wastewater treatment plants and antimicrobial resistance removal

Other than the 35 points across Ireland where untreated wastewater is being discharged into the aquatic environment (EPA, 2020), urban wastewater undergoes primary, secondary and/or tertiary treatment prior to its release into the environment. However, wastewater treatment processes are not designed to eliminate all ARB (DOH, 2017), and this is reflected in the findings of studies recently carried out on wastewater treatment plant (WWTP) influents and effluents in Ireland.

Following completion of the EPA-funded project 'Hospital effluent: impact on the microbial environment and risk to human health', Morris *et al.* (2015) reported that although the impact of wastewater treatment on ARB varies, overall it is evident that current WWTP processes are not capable of removing all ARB/ARGs. As part of this study, the effect of wastewater treatment on AMR *E. coli* was assessed as influent and effluent samples were investigated. Morris *et al.* (2015) reported the presence of AMR *E. coli* in both influent and effluent samples. Although the numbers reduced following wastewater treatment, of particular concern was the persistence of *E. coli* resistant to a wide range of antimicrobials including ampicillin, streptomycin, cefoxitin, cefotaxime, tetracycline, sulphonamide and ciprofloxacin following treatment as such contaminants would subsequently be released into the environment. Morris *et al.* (2015) highlighted the need for further research to better understand the drivers of AMR in WWTPs in relation to development, persistence and dissemination.

A European wide AMR surveillance study of wastewater treatment plant influents and effluents was carried out by Pärnänen *et al.* (2019) in response to one of the AMR global action plan objectives to enhance knowledge on AMR by surveillance and research, and was the first study of its type in Europe. This research was carried out as part of the Water Joint Programming Initiative (JPI)-funded project "Stopping Antibiotic Resistance Evolution" (StARE). Pärnänen *et al.* (2019) compared the resistome of WWTP influent and effluent samples from low antibiotic consumption in humans (LAC) and high antibiotic consumption in humans (HAC) countries, including Ireland, collected throughout 2015 and 2016. However, due to access restrictions in Ireland, only effluent samples were collected and included in this study (Pärnänen *et al.*, 2019). Genes conferring resistance to many commonly used antimicrobials such as aminoglycosides, β-lactams, macrolides, sulfonamides, tetracyclines, phenicols, quinolones, vancomycin and multi drug resistance as well as gene transfer and recombination associated mobile genetic elements (MGEs) were detected in both influent and effluent samples (Pärnänen *et al.*, 2019). With regards to effluent samples, Pärnänen *et al.* (2019) indicated that genes conferring resistance and MGEs were detected in over 90% of samples post treatment, however, an overall reduction in the relative abundance of most ARGs and MGEs was noted. Following treatment, there was a higher relative abundance of most ARGs examined in the effluent of HAC countries, compared to LAC countries with Pärnänen *et al.* (2019) suggesting that WWTP discharges in countries with a HAC, such as Ireland, may have a more significant impact on the receiving aquatic environment.

In order to take a closer look at the AMR status of WWTP effluents in Ireland and investigate the types of ARGs present, the effluents of two WWTPs providing tertiary treatment on all waste collected were assessed by Smyth *et al.* (2020) throughout 2015 and 2016. This work was carried out as part of an EPA and Maynooth University co-funded project 'Survival of mobile antibiotic resistance in water'. Overall, Smyth *et al.* (2020) reported high levels of multi-drug resistance in effluent samples from both WWTPs with faecal coliforms exhibiting resistance to antimicrobials such as aminoglycosides, β-lactams, sulfonamides, tetracyclines, phenicols, fluoroquinolones and colistin (Smyth et al, 2020). Variation in the levels of AMR between both WWTPs was observed and was most likely due to external factors or location. Important findings to note were those relating to β-lactam and colistin in particular. Smyth *et al.* (2020) reported that over 90% of faecal coliforms isolated from WWTP effluents demonstrated resistance to penicillins. Additionally, over 20% of isolates exhibited resistance to cephalosporins. The detection of AmpC producers, as well as extended spectrum β-lactamase (ESBL) producers harbouring ESBL-encoding genes *bla*$_{TEM}$, *bla*$_{SHV,}$ and *bla*$_{CTX-M}$ in effluent samples was also reported. Faecal coliforms resistant to last line antibiotics - carbapenems and colistin – were also found present in the effluent of both WWTPs, however no carbapenemase encoding genes or *mcr* genes were detected.

In contrast to findings by the above studies, Mahon *et al.* (2019) did not detect ARB or ARGs in WWTP effluents. Mahon *et al.* (2019) carried out a study in 2017 investigating WWTP influent and effluent samples from a secondary WWTP and surrounding seawaters in Ireland for the presence of CPE. Although CPE was detected in the surrounding aquatic environment, CPE was not detected in the influent or effluent wastewater samples. However, as study limitations were noted relating to wastewater sample volume and screening methodologies, Mahon *et al.* (2019) highlighted that despite samples testing negative for CPE, WWTP effluent could not be excluded as the possible source of CPE in the receiving environment.

Correlations between wastewater and clinical AMR in Ireland have been reported in recent years. Cahill *et al.*, (2019) detected CPE in hospital wastewater as well as in wastewater collected downstream following entry of hospital wastewater into the municipal stream. The CPE detected reflected those identified in clinical samples in the hospital at the same time. However, some variations were observed suggesting unidentified human carriage of CPE in the healthcare setting. This finding highlighted the potential benefit surveillance of hospital wastewaters may have on monitoring unidentified human carriage of CPE and dissemination in healthcare settings. Similarities between clinical CPE and CPE detected in municipal wastewaters were also reported following comparison of NDM-producing *Enterobacterales* detected in municipal wastewater by Mahon *et al.* (2017) with a collection of clinical CPE previously detected in Ireland. Pärnänen *et al.* (2019) also noted a strong correlation between environmental and clinical AMR as the resistome of urban WWTP samples reflected antibiotic resistance prevalence trends in the clinical setting.

Overall, the majority of these studies highlighted wastewaters including hospital, municipal and WWTP effluents as potentially major sources of AMR in the environment in Ireland. Although a lower abundance of most ARGs post treatment was noted by Pärnänen *et al.* (2019) and a reduction in the number of AMR *E.coli* was reported by Morris *et al.* (2015), these findings are still of concern due to the potential impact the release of effluent containing such contaminants into the environment may subsequently have on human health. Both studies by Pärnänen *et al.* (2019) and Smyth *et al.* (2020) emphasised the need for improved wastewater treatment processes to effectively eliminate ARB and ARGs from entering the environment.

2.1.2 Presence, dissemination and surveillance of antimicrobial resistance in the Irish environment

Antimicrobial resistance in surface waters

In recent years, studies investigating AMR in the aquatic environment have been carried out across Ireland. Among the reviewed research, a range of natural water bodies from surface to ground waters in Ireland were investigated for the presence of AMR.

Two studies carried out in Ireland throughout 2016 and 2017 both reported the detection of CPE in seawater (Mahon *et al.*, 2017; Mahon *et al.*, 2019). Both studies were carried out at separate locations with one assessing a location subjected to untreated wastewater discharges and the other investigating seawaters in the vicinity of a secondary WWTP where no known untreated wastewater was discharged.

Mahon *et al.* (2017) reported the first detection of CPE, an NDM-producing *K. pneumoniae*, in bathing seawaters in both Ireland and Europe in 2017. Initially, Mahon *et al.* (2017) detected NDM-producing *E.coli* in freshwater stream samples in 2016. This then prompted further testing of two freshwater streams, seawater samples from two beaches and as mentioned previously a wastewater system discharging untreated human wastewater in the same locality for the presence of CPE. Seawater and freshwater samples were contaminated with NDM-producing *Enterobacterales* with the source identified as untreated wastewater discharges. Resistance to antimicrobial classes including β-lactams, quinolones, fluoroquinolones, aminoglycosides and tetracyclines was observed among isolates collected from seawater and freshwater samples (Mahon *et al.,* 2017).

A separate, later study carried out by Mahon *et al.* (2019), throughout the 2017 bathing season led to the detection of OXA-48-producing *Enterobacterales* (*E.coli* and *K.pneumoniae*) in seawater samples, despite effluent samples from a nearby WWTP testing negative. However, as previously mentioned in Section 2.1.1, Mahon *et al.* (2019) could not completely exclude WWTP effluents as the source of the CPE contamination due to study limitations. The OXA-48-producing *Enterobacterales* recovered in this study over the course of the bathing season were multidrug resistant, demonstrating resistance to commonly used antimicrobials including β-lactams, quinolones and fluoroquinolones with the OXA-48-producing *K. pneumoniae* also showing resistance to phenicols, aminoglycosides, tetracycline and trimethoprim.

Of particular concern were the findings reported in both studies relating to the correlation between environmental CPE and clinical CPE. When compared to clinical NDM-producing *Enterobacterales*, Mahon *et al.* (2017) reported similarities between the isolates detected in freshwater and seawater and wastewater samples and clinical isolates previously detected in Ireland. NDM-producing *E.coli* detected in one of the freshwater streams and the wastewater system were identical to a clinical isolate detected in Ireland previously. Whereas, the NDM-producing *K. pneumoniae* isolates detected in wastewater and seawater samples, although not identical, were closely linked to a clinical isolate previously detected also. Similarly, the OXA-48-producing *Enterobacterales* detected in seawater samples by Mahon *et al.* (2019) were compared with a collection of clinical isolates previously recovered in Ireland, following which similarities were also noted. The CPE from environmental samples harboured the OXA-48 encoding gene on similar MGEs to clinical CPE isolates. In relation to genetic relatedness, a high degree of similarities were noted among the environmental and clinical OXA-48-producing *E.coli*, however, the OXA-48-producing *K.pneumoniae* from the environment was not closely related to the clinical CPE.

As many studies have focused on and identified wastewaters including WWTP effluents as potential sources of AMR in the environment, Reynolds *et al.*, (2020) investigated other potentially significant sources and reservoirs within the aquatic environment itself. Reynolds *et al.* (2020) investigated small urban streams and connecting bathing waters, none of which received known wastewater discharges, for faecal pollution and ARGs. This work was carried out in Ireland throughout 2017 and 2018 as part of the Acclimatize project which was partly funded by the European Regional Development Fund. Samples from two small urban streams and three bathing water sites in Dublin Bay, into which two of the streams flowed were investigated. Samples from the river Liffey estuary, downstream from the entry point of effluent following secondary wastewater treatment (tertiary during the bathing water season) were also assessed. In addition to investigating the presence of ARGs, levels of faecal indicator bacteria (FIB) as well as human, gull and dog faecal markers were examined in order to determine the potential source of ARG and faecal pollution in these aquatic environments.

Urban streams, bathing waters and the river estuary were all contaminated with high levels of FIB, the human faecal marker, HF183, as well as sulphonamide, tetracycline, fluoroquinolone and β-lactam resistance encoding genes. Although high levels of ARGs were noted in all sample types, the river estuary had the highest abundance of ARGs, followed by streams, with bathing waters having the lowest levels of ARGs. Reynolds *et al.*, (2020) indicated that high levels of faecal contamination and ARGs in the river estuary were most likely due to effluent discharged from the nearby WWTP. However, as the urban streams and bathing waters did not receive any known wastewater discharges, sewer overflows and pipe leaks were suggested as potential sources of contamination. Findings by Reynolds *et al.*, (2020) also indicated that faecal and ARG contamination of the aquatic environment may be fuelled by gull and dog waste, however the authors indicated that further research on their overall contribution to AMR contamination is required. Among all ARGs detected, the sulphonamide resistance encoding gene, *sul1*, present in all sample types, was the most abundant gene detected overall and was most likely due to human faecal contamination. In relation to β-lactam resistance encoding genes, bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M}}$, higher levels were detected in river samples in comparison to streams and bathing waters. Overall, findings reported by Reynolds *et al.*, (2020) highlighted the impact urban streams, despite not receiving any known wastewater discharges, may have on the ARG status of connecting bathing waters.

Reddington *et al.* (2020) also highlighted the importance of surface water surveillance in enhancing our knowledge and developing a greater understanding of microbial diversity including AMR in rivers, as such water bodies are commonly used for recreational, drinking, irrigation and agricultural purposes. Using MinION whole-genome sequencing the metagenomes of 11 rivers worldwide were examined, including the River Corrib in Ireland, throughout 2017 and 2018. In relation to AMR, various mechanisms of antibiotic or multidrug resistance were observed in river metagenomes in this study including the cation/multidrug efflux pump and ABC-type multidrug transport system. Reddington *et al.* (2020) highlighted the potential of using nanopore long-read nucleotide sequencing technology as a way of monitoring river microbiota, particularly in the surveillance of clinically relevant pathogens and ARGs.

The overall findings of the reviewed studies indicated that surface water environments have the potential to act as AMR reservoirs and highlighted the potential role streams and bathing waters may have in AMR dissemination throughout the aquatic environment (Mahon *et al.*, 2017; Mahon *et al.*, 2019; Reynolds *et al.*, 2020). The potential for AMR contamination of the aquatic environment by faecal waste from gulls and dogs was also highlighted and the need for further exploration into the contribution and impact such wastes may have on the environment was indicated (Reynolds *et al.*, 2020). The presence of clinically significant ARB and/or ARGs in surface waters, many of which may confer resistance to multiple antimicrobials, may ultimately pose a serious risk to human health. In order to ensure protection of public health, the need to improve surveillance of surface waters by monitoring AMR routinely was emphasised by findings of the reviewed studies. Routine surveillance would ultimately allow for the development of informed policies and guidelines to ensure water quality, mitigate risk and ultimately safeguard public health.

Antimicrobial resistance in ground waters

In addition to surface waters, ground waters were identified as potential reservoirs of AMR by O'Dwyer *et al.* (2017) following detection of ARB in private groundwater wells in Ireland in 2011 and 2012. All *E.coli* detected were assessed for susceptibility to a panel of commonly used antimicrobials in veterinary medicine as well as to a panel used in human medicine. Overall, *E.coli* resistant to both human and veterinary antimicrobials were detected in groundwater wells. An analysis of causative factors, O'Dwyer *et al.* (2017) identified links between the presence of young children in households and septic tanks and the *E.coli* detected in groundwaters which were resistant to antimicrobials used in human medicine. Livestock density was identified as the potential cause for the presence of *E.coli* resistant to antimicrobials used in veterinary medicine in groundwater samples.

The presence of *E.coli* in groundwaters with resistance to antimicrobials used in human medicine including penicillins, cephalosporins, fluoroquinolones, nitrofurantoin and trimethoprim is concerning due to the potential risk they may pose to human health, especially as these waters may serve as drinking water sources. The findings from this study highlight that groundwaters should not be overlooked as potential reservoirs and transmission routes of AMR. O'Dwyer *et al.* (2017) emphasised the need for further research in this area in order to develop a deeper understanding of AMR prevalence and analyse hydrological and anthropogenic drivers in the Irish subsurface environment.

Investigation of key antimicrobial resistance sources and transmission routes in the aquatic environment in Ireland – a national study

As findings relating to AMR detection in the environment continue to emerge, as previously highlighted, there is a need for increased surveillance of AMR in the environment to inform future policies and ensure protection of public health. In order to carry out monitoring of AMR in the environment and determine measures to reduce contamination and mitigate risk, key sources and transmissions routes must be identified.

For the purposes of a large-scale EPA and Health Service Executive (HSE) co-funded project – '<u>Antimicrobial Resistance and the Environment Sources, persistence, Transmission and risk management project' (AREST)</u> - currently ongoing in Ireland, Chique *et al.*, (2019) used geographic information systems (GIS) and applied a mapping approach to identify potential key sources and transmission routes of AMR in the environment across four local authority areas (LAAs) in Ireland. In the generation of these maps, spatial data relating to population demographics, agriculture, healthcare facilities etc. was analysed. In addition to a wide range of potential sources and transmission routes being identified, these maps also revealed areas where AMR prevalence may potentially be higher. Overall, Chique *et al.*, (2019) highlighted the potential of using GIS to enhance knowledge and understanding of AMR prevalence and spread in the environment, particularly in relation to the 'One Health' context and as a useful tool in selecting relevant sites for monitoring. They also indicated the potential of using this approach to monitor AMR prevalence nationwide and additionally noted the important applications it may have in the identification and analysis of AMR risk and outbreak. This mapping exercise was carried out to ultimately facilitate the AREST project team in the selection of suitable sampling locations across Ireland (Chique *et al.*, 2019).

A nationwide study carried out by Hooban *et al.* (2021) throughout 2018 and 2019, also part of the AREST project, aimed to investigate multiple wastewater sources and natural water bodies for the presence of AMR. Based on maps created by Chique *et al.*, (2019) locations for wastewater and aquatic sampling were selected across the four LAAs in the West, East and South of Ireland. Samples of wastewater from hospitals, long term care facilities (LTCF), airports and WWTPs (influents and effluents) as well as water samples from seawaters, rivers, estuaries, lakes and drinking water treatment plant (DWTP) influents were assessed for the presence of carbapenemase and ESBL – producing *Enterobacterales* and fluoroquinolone resistant *Enterobacterales* over a two-year period.

Wastewater and aquatic environments assessed in this study by Hooban *et al.* (2021) were identified as reservoirs of multi-drug resistant *Enterobacterales*. Overall, a higher percentage of resistant isolates were detected in wastewaters in comparison to waters. Resistance to aminoglycosides, β-lactams, sulfonamides, quinolones, fluoroquinolones, tetracycline, phenicol, trimethoprim, macrolides and fosfomycin was observed in both wastewater and water samples. Of particular concern were the findings relating to β-lactam resistance. Hooban *et al.* (2021) reported on the detection of ESBL and CPE in both wastewater and aquatic environments. Wastewater from hospitals, LTCF, airports and WWTP influents were contaminated with ESBL encoding genes $bla_{\text{CTX-M}}$ and bla_{TEM}, with bla_{SHV} found in hospital and WWTP influents also. The presence of OXA-48 producing CPE in WWTP influents was similar to findings by Cahill *et al.* (2019) n hospital wastewater samples. With regards to the aquatic environment, similar to Reynolds *et al.* (2020), the presence of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ in seawater and river/estuary samples was also reported by Hooban *et al.* (2021). Additionally, *bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ were found present in lake samples and *bla*$_{CTX-M}$ in DWTP samples. In relation to CPE, *bla*$_{OXA-48}$ was detected in estuary samples, *bla*$_{NDM}$ in lakes, and in seawaters carbapenemase encoding genes detected included *bla*$_{OXA-48}$ and *bla*$_{NDM}$, both of which have previously been detected in Irish seawaters by Mahon *et al.* (2017) and Mahon *et al.* (2019). The NDM variant detected, NDM-5, was previously detected by Cahill *et al*. (2019) in municipal wastewaters.

Correlations between isolates recovered from wastewater and water samples were noted by Hooban *et al.* (2021) and highlighted the potential persistence and dissemination of clinically relevant ARB and ARGs from wastewaters to and within the aquatic environment. Other than widespread dissemination of AMR contaminants being a potential cause of their presence in aquatic environments receiving minimal or no anthropogenic pollution, Hooban *et al.* (2021) also indicated that the presence of ARB and/or ARGs in such environments may also reflect the natural resistome of the environment.

As there is little knowledge on the impact aquatic environments contaminated with ARB and ARGs may ultimately have on human health, the need for further research in this area was highlighted. Overall, findings by Hooban *et al.* (2021) and many of the reviewed studies, highlight the need for nationwide routine surveillance of AMR in the Irish aquatic environment and a harmonised approach to same. This is particularly true as limitations in the current EU bathing water monitoring criteria were highlighted as bathing waters which had been classified as good/excellent quality were found to be contaminated with resistant organisms and genes.

2.2 Antimicrobials in the Environment in Ireland

Antimicrobials in the environment are increasingly recognised as emerging contaminants of concern. The environment including soils, plants, surface waters, ground waters etc. is at risk of receiving antimicrobials from a variety of different sources for example via wastewaters, manure, land spreading of biosolids, as well as through landfill leachate or industrial waste emissions (DOH, 2017). The entry and spread of antimicrobials in the environment is increasingly concerning due to the potential impact their presence may have on the acceleration of AMR in the environment and the subsequent impact on human and animal health. Studies relating to the potential sources and detection of antimicrobials in the environment in Ireland have been published in recent years.

2.2.1 Potential sources of antimicrobials in the Irish environment

In addition to ARB, hospital and municipal wastewaters, as well WWTP influents in Ireland were investigated for the presence of antimicrobial residues as part of the EPA-funded project 'Hospital effluent: impact on the microbial environment and risk to human health' (Morris *et al.*, 2015). Hospital wastewaters which are hotspots for antimicrobial residues as a result of large-scale AMU in hospitals were reported as a potential contributor of antimicrobial residues to municipal wastewaters. Residues of both penicillins and quinolones were detected in hospital wastewaters as well as municipal wastewaters receiving hospital waste. However, concentrations were found to reduce following their release into the municipal wastewater stream, possibly due to factors such as dilution and biotransformation, with no residues of antimicrobials being detected in WWTP influent samples assessed.

In another study carried out as part of the same project, fluoroquinolones were identified, by use of a Monte Carlo simulation model, as antimicrobials of great concern and potential AMR drivers in the environment due to their low rate of degradation and level of toxicity (Morris *et al.*, 2015). This model was also used to predict the fate of ciprofloxacin in the environment following wastewater treatment. Although concentrations of this antimicrobial were predicted to be low in the receiving environment and pose a low risk to water users, Morris *et al.* (2015) highlighted that its presence in the environment may still pose a threat to the microbial ecosystem and potentially contribute to AMR development.

As WWTP processes are not designed to effectively eliminate all antimicrobial residues (DOH, 2017), these contaminants can persist following wastewater treatment, in sewage sludges (Healy *et al.*, 2017a) and final effluents (Rodriguez-Mozaz *et al.*, 2020).

In 2019, 89% of treated sewage sludge in Ireland was used in agriculture as a soil enhancer or fertiliser (EPA, 2020) . If antimicrobial residues are present in these biosolids there is a risk of transfer to plants, soils, ground waters or through runoff into surface waters. Therefore, there are concerns around land application of biosolids and the potential contribution they may have to the emergence and dissemination of AMR in the environment. Healy *et al.* (2017a) published the findings of a study carried out in 2015 which investigated sewage sludges in 16 WWTPs across the country for the presence of the antimicrobials triclosan (TCS) and triclocarban (TCC) and following land application, their presence in surface runoff. This study was the first in Ireland to assess the concentrations of TCS and TCC in

WWTP sludges and was carried out as part of an EPA-funded project 'Health and Water Quality Impacts Arising from Land Spreading of Biosolids' (Healy *et al.*, 2017b). Overall, Healy *et al.*, (2017a) reported low concentrations of both antimicrobials in WWTP sludges and surface runoffs, therefore indicating that surface runoff was not a major source of these antimicrobials in the environment. The restrictions relating to the use of TCS in recent years and reductions in the use of TCC by pharmaceutical industries were highlighted as potential reasons for the overall low concentration of both in biosolids assessed in this study. However, as this study only focused on two antimicrobials in particular, higher concentrations of others may exist. In addition, many years of land spreading biosolids may result in an accumulation of antimicrobials in the soil which may subsequently spread to the surrounding environment. This, coupled with the uncertainty that still exists around the public health risk associated with the land spreading of biosolids highlights the need for further investigations in this area.

With regards to WWTP final effluents, antimicrobial residues were detected in samples from 13 WWTPs across seven European countries, including Ireland, throughout 2015 and 2016 by Rodriguez-Mozaz *et al.* (2020). This trans-European surveillance study was the first of its type and was funded through the EPA in Ireland under the Water JPI project StARE. Residues of fluoroquinolones, cephalosporins, lincosamides, macrolides, nitroimidazole, trimethoprim, penicillins, quinolones, sulfonamides, and tetracycline were detected in effluent samples. Although variations in antimicrobial concentrations were observed across all WWTPs and countries, Rodriguez-Mozaz *et al.* (2020) found antimicrobial concentrations in effluent samples generally reflected levels of human AMU in individual countries. Ireland, Spain and Portugal, all countries of high human AMU had the highest antimicrobial concentrations in effluent samples overall. In addition to effluents, Rodriguez-Mozaz *et al.* (2020) suggests that future studies should also assess influents in order to gain a greater understanding of the link between AMU and antimicrobial presence in WWTPs.

Despite low concentrations of antimicrobials being detected in some WWTP effluents by Rodriguez-Mozaz *et al.* (2020) and in biosolids and surface runoffs by Healy *et al.*, (2017a), as also previously highlighted by Morris *et al.* (2015), the presence of certain antimicrobials in the environment may still impact microbial populations and potentially contribute to the development of AMR through selective pressure. Overall, findings by both Healy *et al.* (2017a) and Rodriguez-Mozaz *et al.* (2020) highlighted the need for further monitoring of antimicrobials in WWTPs. However, as outlined by both, due to high costs it would not be feasible to measure the concentrations of all antimicrobials. Both suggested focusing on those of primary concern with Healy *et al.*, (2017a) recommending testing of TCS and TCC in biosolids and Rodriguez-Mozaz *et al.* (2020) recommending the use of ciprofloxacin, azithromycin, and cefalexin compounds as potential markers of antimicrobial contamination in WWTP effluents or the aquatic environment.

Morris *et al.* (2015) highlighted the need for the harmonisation of international antimicrobial surveillance in the environment through development and implementation of guidelines as well as the development of standardised methods relating to the sampling, testing and reporting of antimicrobials in the environment including wastewaters and their receiving environments. However, as indicated, the development of standardised methods could prove quite challenging due to the wide range of antimicrobials which exist. Therefore, Morris *et al.* (2015) recommend initially focusing on the commonly used antimicrobials known to persist in the environment. In addition, when selecting antimicrobials of priority for monitoring, their rate of metabolism should also be taken into consideration as those with lower rates of metabolism in the body are more likely to persist and subsequently enter the environment. Morris *et al.* (2015) highlighted the potential of using models such as the Monte Carlo model to assess the risk of AMR development by predicting antimicrobial concentrations in the environment.

2.2.2 Antimicrobials in the Irish aquatic environment

In recent years, little research has been published on studies assessing the aquatic environment in Ireland for the presence of antimicrobials. However, some work has been carried out on the development of methods for the determination of antiparasitic drugs used in veterinary medicine, in particular, anthelmintics (Mooney *et al.*, 2019) and anticoccidials (Mooney *et al.*, 2020a), in the aquatic environment. In addition to the development of these methods, studies were carried out using these methods to monitor the occurrence of such contaminants in both surface and subsurface environments in Ireland (Mooney *et al.*, 2019; Mooney *et al.*, 2020b). All of these works were carried out as part of the Groundwater Challenge of the Irish Centre for Research in Applied Geosciences (iCRAG) and funded through the Teagasc Walsh Scholarship programme.

Mooney *et al.* (2019) developed and validated a method for the detection of 40 anthelmintic compounds in both surface and subsurface waters in Ireland. This work was co-funded under the European Regional Development Fund and by iCRAG industry partners and was carried out due to the lack of knowledge around the presence and concentrations of anthelmintics in the environment. Although methods had been developed previously for the determination of anthelmintics in the environment, such methods only focused on a limited number of compounds and often from only one class, whereas this particular method enables the detection of a wide range of such compounds from a variety of classes. This particular method involves the extraction of anthelmintic compounds from raw samples by solid phase extraction followed by liquid chromatography tandem mass spectrometry detection and was validated at a range of concentrations in line with those expected to be present in the aquatic environment. A study to assess this multiresidue detection method was carried out by Mooney *et al.* (2019) and involved the investigation of both surface (n=20) and subsurface (n=52) water samples collected from various sites across Ireland for the 40 anthelmintic compounds, in 2016. This was the first study of its type according to Mooney et al (2019) and resulted in the detection of anthelmintic residues at concentrations ranging from 1.0 ng L−1 to 30 ng L−1 in both surface and subsurface environments with 20% of surface waters and 7.7% of ground waters assessed contaminated with such residues.

Overall, this method was deemed suitable and accurate for the detection of a wide range of anthelmintic compounds and their transformation products in the aquatic environment. According to Mooney *et al.* (2019), the use of this method in the investigation of both surface and subsurface waters for the presence of anthelmintic compounds will provide extensive insight into and therefore better our understanding of the presence and fate of anthelmintics in such environments.

This method was later applied in further studies carried out by this group in Ireland throughout 2017 and 2018, where both spatial occurrence and temporal variations were investigated (Mooney *et al*., 2021). Due to their common use in livestock farming and lower rates of metabolism, Mooney *et al*. (2021) highlighted anthelmintics and their transformation products as contaminants of emerging concern and therefore indicated the need for further investigation of the aquatic environment for these contaminants. Mooney *et al*. (2021) assessed subsurface waters (n=88) across Ireland for the presence of anthelmintic compounds and their transformation products and to complement subsurface sampling a small number of surface waters (n=18) were also investigated. Sampling took place in areas of karst and fractured bedrock aquifers due to the potential higher risk of subsurface contamination in these particular areas. The detection of anthelmintic residues in both surface (39%) and subsurface waters (18%) with concentrations up to 41 ng L−1 were reported. Although a higher percentage of surface water sites were contaminated in comparison to subsurface water sites, a wider range of anthelmintic drugs was detected in subsurface waters where sixteen different compounds were present, in comparison to eight in surface waters. Overall, the most prevalent anthelmintic in both surface and subsurface water environments was albendazole, a commonly used benzimidazole drug. Mooney *et al*. (2021) reported on several factors including tillage farming, livestock densities, in particular, sheep and soil type, which they believe in combination may have an influence on the occurrence of anthelmintics in subsurface waters. The results of the temporal study revealed that both biosolid land application and animal return to pasture can impact anthelmintic occurrence in subsurface waters as an increase in occurrence was noted during these times. In addition, Mooney *et al*. (2021) indicated that meteorological factors and anthelmintic usage can also influence anthelmintic occurrence. According to Mooney *et al*. (2021) more research is required in this area, particularly to establish quality standards for these contaminants in the aquatic environment.

In addition to anthelmintics, this particular research group also developed and validated a method for the determination of anticoccidials (6 ionophores and 20 synthetic anticoccidials) in the aquatic environment, including those present at very low concentrations (Mooney *et al.*, 2020a). Similar to the method developed for the determination of anthelmintics by Mooney *et al.* (2019), anticoccidial residues were extracted from raw water samples by solid phase extraction, eluted, concentrated and detected by liquid chromatography tandem mass spectrometry. This particular method was also validated at a range of four different concentrations and deemed successful for the detection of these 26 anticoccidials in environmental samples. Prior to the development of this method by Mooney *et al.* (2020a), there was a lack of comprehensive analytical methods for the detection of such compounds in both surface and subsurface environments therefore resulting in limited research being carried out and knowledge around the presence and fate of anticoccidials in the environment. Similar to anthelmintics, many previously developed methods focused on a smaller range of anticoccidials. When compared to previously developed methods, this method developed by Mooney et al. is more useful due to improved performance, better detection capabilities, as well as its ability to simultaneously detect a wide range of anticoccidials including highly polar compounds (Mooney *et al.*, 2020a).

This particular method was used in a study completed in 2018, investigating groundwaters across Ireland for these veterinary anticoccidials (Mooney *et al.*, 2020b). This study reported the first detection of anticoccidials in groundwaters in Ireland, as 7 out of the 26 investigated were detected across 26 of the 109 sampling points assessed. Anticoccidials detected included lasalocid, monensin, narasin, salinomycin, amprolium, diclazuril and nicarbazin, with monensin and amprolium being the most commonly detected overall. Both are commonly used in poultry production, with monensin as a feed additive and amprolium as a medicine. Mooney *et al.* (2020b) reported poultry activity i.e. farming or manure spreading as a major driver behind anticoccidial presence in groundwaters as such activity was present in the surrounding area of all but two of the groundwater sites where anticoccidial compounds were detected in this study.

Mooney *et al.* (2020b) also reported on the potential links between the occurrence of anticoccidials in groundwaters and the acidity of surrounding soils as well as calcium, conductivity, ammonium, faecal coliforms and a lower groundwater pH in the subsurface environment. The need for further research was highlighted in order to enhance our knowledge in this area and draw conclusions on whether in fact there is a definite relationship between anticoccidial occurrence in groundwaters and the above factors. Other factors which may have had an influence on the presence of anticoccidials in groundwater, such as rainfall or timing of manure land spreading were not assessed in this study, however as indicated, should be taken into account in future studies.

In addition to Mooney *et al.* (2020b) reporting on the first detection of anticoccidials in groundwaters in Ireland, they also reported the first detection of the anticoccidials lasalocid, narasin, salinomycin, amprolium, diclazuril and nicarbazinin in groundwaters in Europe. Overall, the findings of this study emphasised the need for the incorporation of anticoccidial surveillance in groundwater quality monitoring programmes. The study highlighted anticoccidials which may be of greatest concern as well as particular areas which may be vulnerable to anticoccidial contamination and therefore may need to be taken into consideration when monitoring for anticoccidials in the aquatic environment.

2.3 Conclusion

It is evident from the above reviewed studies carried out in Ireland that both untreated and treated wastewaters as well as surface and subsurface environments are potential sources and reservoirs of AMR and antimicrobial residues. As current wastewater treatment processes do not have the ability to eliminate all antimicrobials and AMR contaminants, the release of effluents into the aquatic environment or the use of sewage sludges in agriculture may result in AMR development, emergence and dissemination in the environment. Although concentrations may reduce following wastewater treatment, the presence of such contaminants may still drive AMR in the environment and ultimately pose a risk to human or animal health.

In order to enhance our understanding of AMR presence, persistence and dissemination in the environment further work in this area is required in Ireland. Overall, as highlighted throughout this review there is a major need for routine harmonised surveillance of both surface and subsurface environments for the presence of AMR and antimicrobial residues. The development and implementation of standardised methodology for sampling, testing and reporting of antimicrobials and AMR contaminants in the environment is required. Both GIS and whole genome sequencing technologies have been highlighted as useful tools in the identification of potential sampling points and the monitoring of such environments. In addition to surveillance, further research is required to assess AMR drivers and factors which may contribute to or influence the presence and persistence of AMR in the environment. In relation to WWTPs, in order to improve treatment processes and infrastructure to ensure effective elimination of antimicrobials and AMR contaminants there is a need for further research to better understand the role WWTPs play in the development, persistence and dissemination of AMR. Overall, further research and surveillance of AMR in the environment in Ireland is necessary, particularly for the development and implementation of appropriate regulations and measures to reduce AMR dissemination, mitigate risk and ultimately safeguard public health.

3. Ongoing Research on Antimicrobial Resistance and the Environment in Ireland

Projects currently ongoing in relation to AMR and the natural environment in Ireland are listed in Table 3.1 below. Research relating to sources, hotspots, drivers, transmission and impact of antimicrobial and AMR contaminants in the environment, as well as the development of detection tools and risk management protocols is being conducted under the listed projects.

Table 3.1 Projects currently ongoing in Ireland and funding organisations

Project	Funding Organisation
AREST - Antimicrobial Resistance and the Environment – Sources, persistence, Transmission and risk management	EPA/HSE Co-fund
ANTIVERSA - Biodiversity as an ecological barrier for the spread of clinically relevant antibiotic resistance in the environment	EPA/ European Commission Co-fund
PIER - Public health Impact of Exposure to antibiotic Resistance in recreational waters	EPA
Analysis of antimicrobial resistance in private water drinking supplies	EPA/ Maynooth University Co-fund
SWAM - Survival of mobile antibiotic resistance in water	EPA/ Maynooth University Co-fund
INART - Intervention of antimicrobial resistance transfer into the food chain	HRB funded JPIAMR

Citizens and Science Workshops on Antimicrobial Resistance in Private Drinking Water in Ireland	IRC New Foundations
Exploring Irish soil as a reservoir for antimicrobial resistance	SFI /GSI
SMARTIE - Spatiotemporal multiscale Modelling of Antimicrobial Resistance in The Irish subsurface Environment	iCRAG/ GSI Co-fund
WORLDCOM: Development of new tools for real-time detection of zoonotic bacteria and antimicrobial resistance in veterinary, human and environmental sources	One Health EJP
FED-AMR: The role of free extracellular DNA in dissemination of antimicrobial resistance over ecosystem boundaries along the food/feed chain	One Health EJP
DiSCoVer: Discovering the sources of Salmonella, Campylobacter, VTEC and antimicrobial resistance	One Health EJP
Comparative risk of antimicrobial resistance transfer from poultry, pig and bovine manure to grassland	Teagasc
Investigating the role of heavy metals in the environment as a selective pressure for the dissemination of antimicrobial resistance	One Health EJP / Teagasc Co-fund

EPA, Environmental Protection Agency; HSE, Health Service Executive; HRB. Health Research Board; JPIAMR, Joint Programming Initiative on Antimicrobial Resistance; IRC, Irish Research Council; SFI, Science Foundation Ireland; iCRAG, Irish Centre for Research in Applied Geosciences; GSI, Geological Society Ireland; OHEJP, One Health European Joint Programme

4. Identification of Key Knowledge Gaps Worldwide

On a global level, knowledge gaps relating to AMR and the environment have been identified and reported on in recent years by Larsson *et al.* (2018). This work which was funded under Horizon 2020, involved the distribution of a questionnaire, followed by a workshop with key experts in the area to discuss and identify existing knowledge gaps relating to the environmental dimension of AMR. Although many knowledge gaps exist, Larsson *et al.* (2018) identified key areas where research was urgently required. These include;

- The sources of AMR and how these sources contribute to the development and spread of antimicrobials and AMR contaminants in the environment. The importance of developing standardized methods for the testing and reporting of antimicrobials and AMR contaminants was also highlighted.

- The role of the environment and the impact of anthropogenic pollution on the evolution of AMR.

- The impacts of exposure to AMR in different environmental compartments on human and animal health.

- Effective interventions to reduce dissemination and mitigate risk of AMR in the environment, including social, behavioural, economic and technological interventions.

As indicated by Larsson *et al.* (2018), it is essential that we take into consideration and address the key knowledge gaps through future research in order to expand our understanding around AMR emergence, spread and the risks associated as well as to develop and implement effective control and mitigation measures.

In Ireland, although research has been carried out previously and is ongoing relating to AMR and the environment, knowledge gaps still exist (DOH, 2017). The need to take a One Health approach to adequately address the challenge of AMR is acknowledged in *i*NAP. In development of *i*NAP2 an increased emphasis on our understanding of the role of the environment in the persistence and transmission of AMR is essential. Research is required to increase our understanding of the environmental dimension of AMR to effectively inform future policies and strategies relating to the management and prevention of AMR. (DOH, 2017).

In order to determine research needs for the coming years in Ireland, key knowledge gaps in this area were identified through;

- A poll delivered at the EPA, HSE and The Economic and Social Research Institute (ESRI) 'Environment, Health & Wellbeing' virtual conference, November 26th 2020.

- The circulation of a survey on AMR and the environment to key researchers involved in the AMR field in human, animal and environmental sectors in Ireland.

- A key stakeholder workshop relating to AMR and the environment which included representatives from research institutions, funding organisations and government bodies.

Findings of these processes are outlined in Sections 5 and 6. There is a need for further investments in this area to be made at a national level.

5. Poll and Survey Distribution and Results

Based on the key knowledge gaps identified by Larsson *et al.* (2018), a poll and survey were developed.

5.1 Live Poll

A poll was created and put forward to the audience attending the ‘Environment, Health & Wellbeing’ virtual conference hosted by the EPA, HSE and the ESRI on November 26th, 2020. The audience (n=275) included a wide range of representatives from human, animal, agriculture, food and environmental health and science sectors, as well as members of the general public. Attendees were asked to select the area in which they believe the key knowledge gaps exist in relation to the environmental dimension of AMR in Ireland. Although all areas were identified as being knowledge gaps by those who responded to the poll, the impact of exposure to AMR on human health and effective interventions to mitigate risk were identified as two of the key areas where knowledge gaps currently exist, by 41% and 39% of participants, respectively (Figure 5.1).

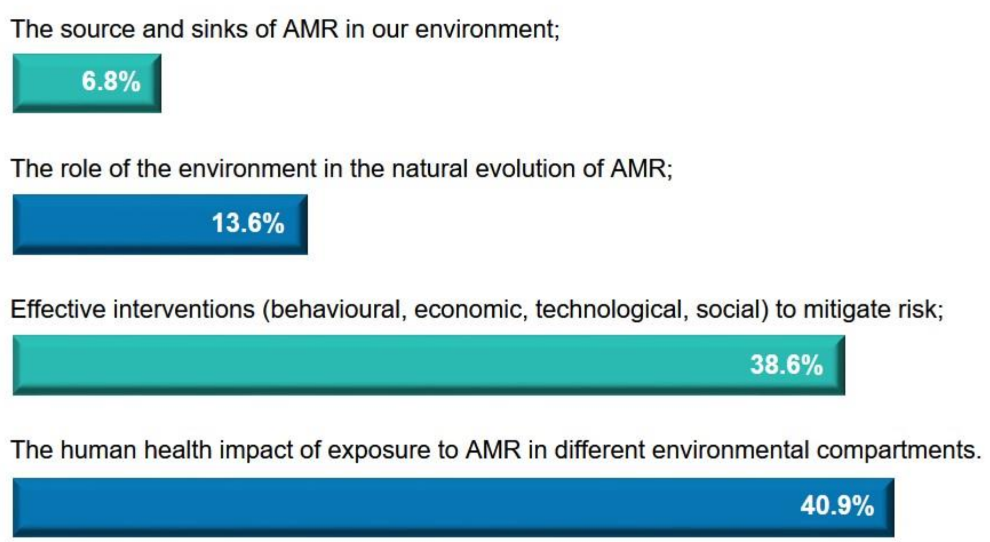


Figure 5.1. Live poll results indicating the key knowledge gaps in the environmental dimension of AMR.

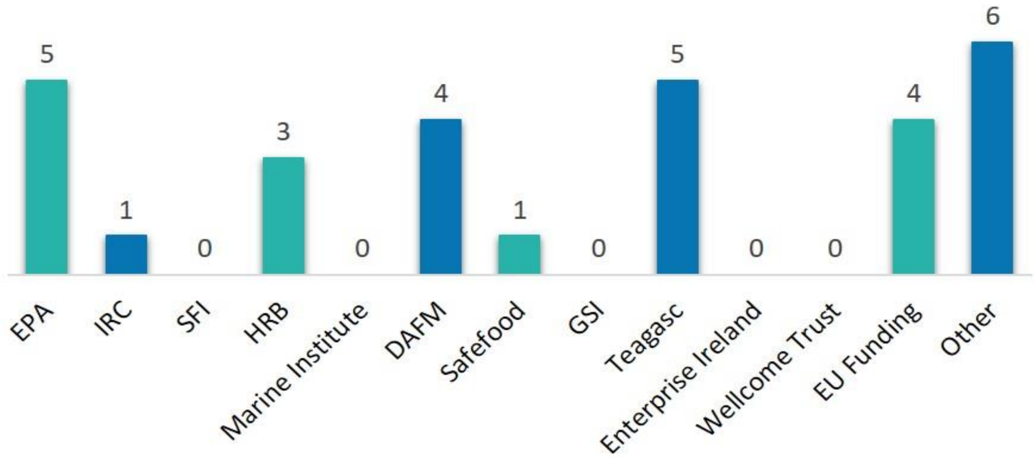
5.2 Survey

5.2.1. Development and distribution

In order to fully understand and gain a more in-depth insight into the key knowledge gaps which exist, a survey was developed and distributed in March 2021. The survey aimed to identify the key knowledge gaps in relation to the sources, reservoirs and transmission routes of AMR, the human, animal and environmental impact of AMR, as well as the role of the environment in the evolution of AMR and effective interventions to mitigate risk. See Appendix 1 for details of the survey distributed. This survey was developed specifically for distribution to researchers who have previously worked, or are currently working, in the area of antimicrobials and AMR in human, animal and environmental sectors in Ireland. The survey was created using the Office 365 application 'Microsoft Forms' and distributed via email to 78 researchers from a wide range of research organisations, including; Athlone Institute of Technology, Cork Institute of Technology, Dublin City University, Limerick Institute of Technology, Maynooth University, National University of Ireland Galway, Royal College of Surgeons Ireland, Teagasc, Technological University Dublin, Trinity College Dublin, University College Cork, University College Dublin, University of Limerick and Waterford Institute of Technology.

5.2.2 Results

Of the 78 researchers to which the survey was distributed, it was completed anonymously by 22, potentially reflecting the limited number of researchers currently active in the AMR and environment field in Ireland. Fifteen respondents indicated that they had been involved in research relating to AMR and the environment in the last five years, working in areas such as AMR in soil, surface waters, groundwaters, wastewaters, manure transfer, plants (both edible and non-edible), as well as areas relating to the farm and built environments. Figure 5.2 indicates the various organisations from which respondents (n=14) received funding for AMR related research projects in the last five years. Six respondents indicated that they had received funding from 'Other' sources including, The Swiss National Science Foundation (n=1), The Government of Saudi Arabia (n=1), University Funding (n=1), Dublin Dental University Hospital Microbiology Department (n=1), The Medical Research Council (United Kingdom) (n=1), while the remaining one respondent had not received any funding for the research carried out.



EPA, Environmental Protection Agency; IRC, Irish Research Council; SFI, Science Foundation Ireland; HRB, Health Research Board; DAFM, Department of Agriculture, Food and the Marine; GSI, Geological Society Ireland.

Figure 5.2. Organisations from which respondents received funding for research relating to AMR in the environment.

Sources and reservoirs of antimicrobial resistance

In relation to the sources of AMR in the environment, participants ranked waste sources in order of what they believed were the least investigated to most investigated in Ireland (Figure 5.3). Over half of respondents (54%; n=12) indicated that waste from wildlife was the least investigated source of AMR in the environment in Ireland. Companion animals were ranked in second place overall, however as indicated by two respondents, while wildlife and companion animals may be the least investigated sources of AMR they may not necessarily be the most important sources to investigate. Overall, human waste was highlighted as the most investigated source of AMR in the environment, with 40.9% (n=9) of respondents ranking this waste source in seventh place. However, one respondent highlighted wastewater from healthcare facilities as a potential source of AMR and its contribution to AMR in the environment as an area requiring further investigation.

Figure 5.3. Ranking of sources of AMR in the environment, from least investigated (1) to most investigated (7).

With regards to environmental reservoirs for AMR, plants, both non-edible and edible, were identified as areas which have been least investigated to date (Figure 5.4). In relation to non-edible plants, 38.9% (n=7) of respondents ranked these as the least investigated area. On the other hand, freshwater was identified as the most investigated reservoir, according to 33.3% of respondents (n=6). In addition, a number of participants indicated aerosols or airborne particles as another potential reservoir for AMR in the environment which may also be worth investigating. In addition to the those outlined in Figure 5.4, other potential reservoirs of AMR in the environment which may require further research indicated by respondents included animal by-products e.g. bones and biological organisms e.g. invertebrates.

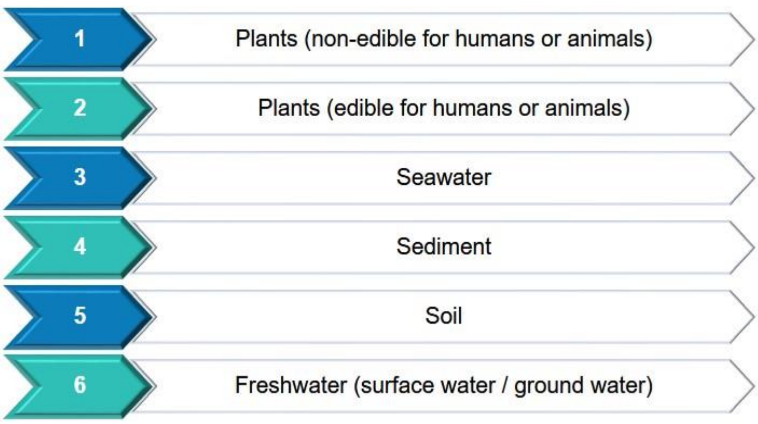


Figure 5.4. Ranking of environmental reservoirs of AMR, from least investigated (1) to most investigated (6).

The role of the environment in the natural evolution of antimicrobial resistance

Overall, 73% (n=16) of respondents indicated that they believe the natural environment is the original source of many ARGs in human and animal pathogens. In order to understand the role of the natural environment in the evolution of AMR of human and animal health importance, respondents indicated a variety of areas they believed required further research. The need for investigation of AMR evolution from source to final receptor was highlighted. Sources, reservoirs and transmission routes of ARGs including soils, sediments, wastewaters and waters such as surface, irrigation and drinking were also all indicated as areas in need of further research. In relation to the investigation of soil microbiota, respondents highlighted the need for further studies to explore less contaminated soils as well as different factors such as time, acidity, hydration, erosion, soil amendments and variability of trace elements. A number of respondents also commented on the need for investigations into drivers of AMR selection and persistence in the environment. The need for further research on the spread of ARGs within environmental bacteria and also to both human and animal pathogens as well as the factors promoting the mobilisation of these ARGs was also highlighted. The role of gene sharing in pathogen evolution was another area indicated by respondents as a key knowledge gap in relation to AMR and the environment.

Effective interventions to mitigate risk of antimicrobial resistance in the environment

With regards to effective interventions to mitigate the risk of AMR in the environment, the order in which respondents ranked the least investigated to most investigated interventions is shown in Figure 5.5. Nine respondents (42.9%) indicated behavioural interventions (social/personal) such as antimicrobial stewardship, personal responsibility/behaviours, as the least investigated area to date. To ensure appropriate use of antimicrobials, one respondent indicated the need for a national electronic prescription system for all veterinary medicines and zootechnical feed additives.

Technological interventions e.g. waste management and wastewater treatment systems were ranked in second place. In relation to waste treatment, one respondent highlighted that further work needs to be carried out in relation to the treatment of healthcare effluents and livestock slurry. Another identified AMR evolution and antimicrobial residue biodegradation in wastewater treatment systems as areas they believe warrant further investigation. The use of constructed 'bio-barriers' in the environment to disrupt AMR conduits was also highlighted as a potential intervention to mitigate risk and an area to be explored.

According to 47.6% (n=10) of respondents, economic interventions e.g. development and enforcement of policies and standards around appropriate antimicrobial use, are the most investigated to date.

Ecological interventions were also indicated by one respondent as an additional area to explore in relation to effective interventions to mitigate the risk of AMR in the environment. Taking into account the 'One Health' context, the need for inter-disciplinary research projects involving human, animal and environmental sectors, looking at behavioural interventions in combination with technological interventions, was also highlighted by another respondent.

In relation to interventions to reduce the risk of AMR, additional areas in which respondents indicated further research is required included biological evolution as well as methods of inhibiting transmission at a molecular level.

Figure 5.5. Ranking of effective interventions to mitigate risk of AMR in the environment, from least investigated (1) to most investigated (3).

Transmission of antimicrobial resistance between the environment, humans and animals

In terms of transmission of AMR, respondents were asked to rank, from least investigated to most investigated, the routes between the environment and humans, the environment and animals, as well as the routes within the environment itself.

Transmission between air and humans was identified as the least investigated transmission route between the environment and humans (Figure 5.6) with 38.1% (n=8) of respondents ranking this route in first place. This was followed in order by seawater, plants, soil and freshwater as transmission routes of AMR between the environment and humans. The freshwater and human transmission route was found to be overall the most investigated route to date between the environment and humans, with one third of respondents (33.3%; n=7) ranking this this route in fifth place.

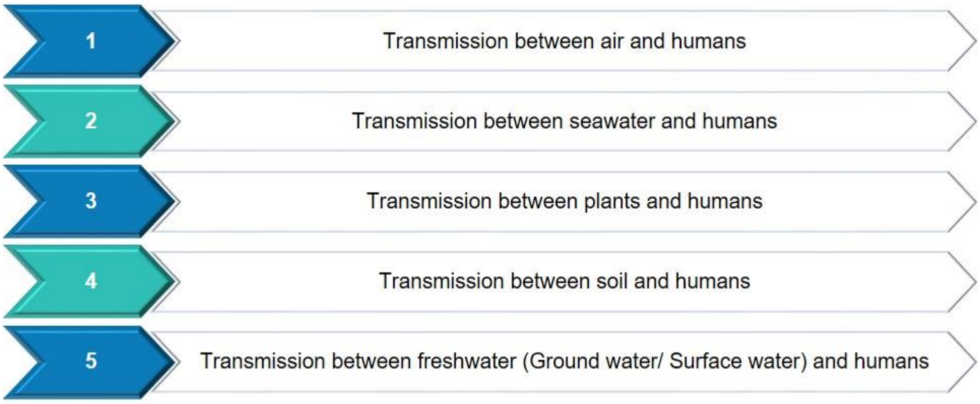


Figure 5.6. Ranking of AMR transmission routes between the environment and humans, from least investigated (1) to most investigated (5).

Similarly, the order in which transmission routes between the environment and animals were ranked mirrored that of the environment and humans as respondents ranked the routes of transmission from least investigated to most investigated in the order of air, seawater, plants, soil and freshwater (Figure 5.7). Transmission between air and animals was identified as the least investigated, with 42.1% (n=8) of respondents ranking this route in first place. Transmission between freshwater and animals was identified as the most investigated, with 47.4% (n=9) of respondents ranking this route in fifth place.

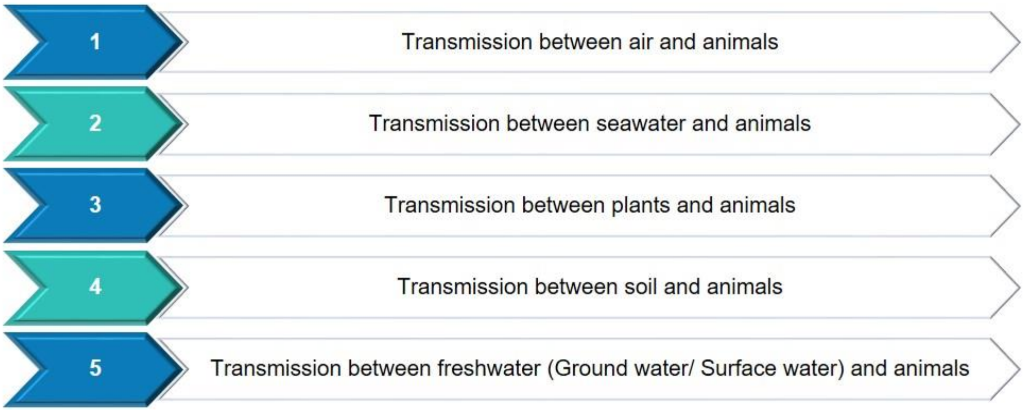


Figure 5.7. Ranking of AMR transmission routes between the environment and animals, from least investigated (1) to most investigated (5).

With regards to transmission routes within the environment, overall air was the area identified as least investigated with transmission between air and seawater identified as the least investigated route by 50% (n=8) of respondents, followed by air and plants, soil and freshwater (Figure 5.8). Other than transmission between air and freshwater, all other routes between the freshwater environment and other environmental compartments including plants, seawater and soil were suggested as the most investigated to date. The freshwater to soil transmission route was overall found to be the most investigated area with 50% (n=8) of respondents ranking this route in tenth place.

Figure 5.8. Ranking of AMR transmission routes within the environment, from least investigated (1) to most investigated (10).

According to 89.5% (n=17) of respondents, transmission routes of AMR within the environment are those least investigated overall, followed by transmission routes between the environment and animals (Figure 5.9). Routes between the environment and humans were identified as most investigated to date according to 63.2% (n=12) of respondents. In relation to the environment and AMR transmission, one respondent felt investigations into the relative importance of the environment compared to other routes of transmission were needed. In addition to the transmission routes already outlined, one respondent also highlighted extracellular DNA as a distinct route for transmission requiring further research.

Figure 5.9. Ranking of the knowledge gaps relating to the transmission of AMR between the environment, humans and animals, from least investigated (1) to most investigated (3).

The human, animal and environmental health impact of exposure to antimicrobial resistance in different environmental compartments

In relation to the impact of exposure to AMR, key knowledge gaps were ranked by survey participants in order from least investigated to most investigated (Figure 5.10). The risk of disruption to the natural biodiversity in the environment was identified by 80% (n=16) of respondents as the area least investigated to date. This was then followed by the risk of colonisation with antimicrobial resistant organisms for both animals and humans, with the risk of infection for animals and humans considered the areas most investigated. The risk of infection with antimicrobial resistant organisms for humans was identified as the area most investigated to date according to 40% (n=8) of respondents. One respondent also indicated the need for investigations into how a combination of factors including environmental, social and economic factors can affect the risk of exposure to AMR.

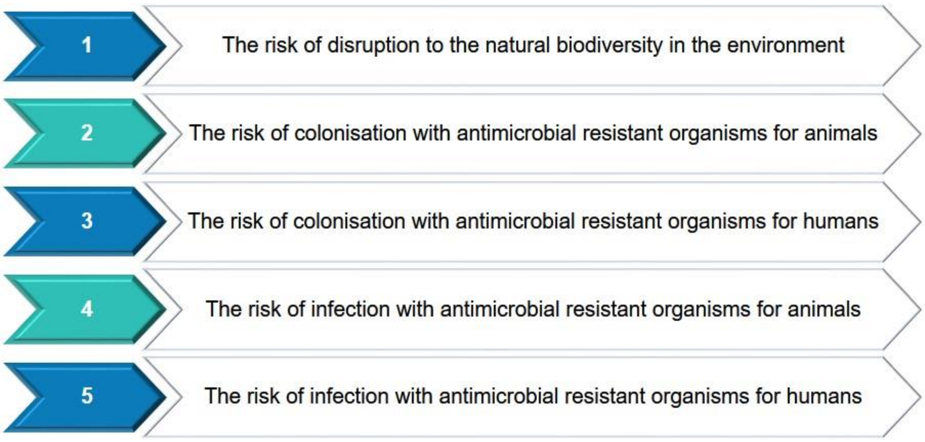


Figure 5.10. Ranking of the knowledge gaps relating to the impact of exposure to AMR in different environmental compartments on human, animal and environmental health, from least investigated (1) to most investigated (5).

Overall key knowledge gaps identified and areas of additional research

Respondents ranked the areas they believed were the key areas where knowledge gaps existed in the environmental dimension of AMR at present In Ireland (Figure 5.11). The role of the environment in the natural evolution of AMR was considered the least investigated area, with 31.8% (n=7) of respondents ranking this area in first place, while the sources and reservoirs of AMR in our environment was found to be the most investigated area overall. However, one respondent indicated the difficulty in ranking these knowledge gaps as many gaps exist in all areas.

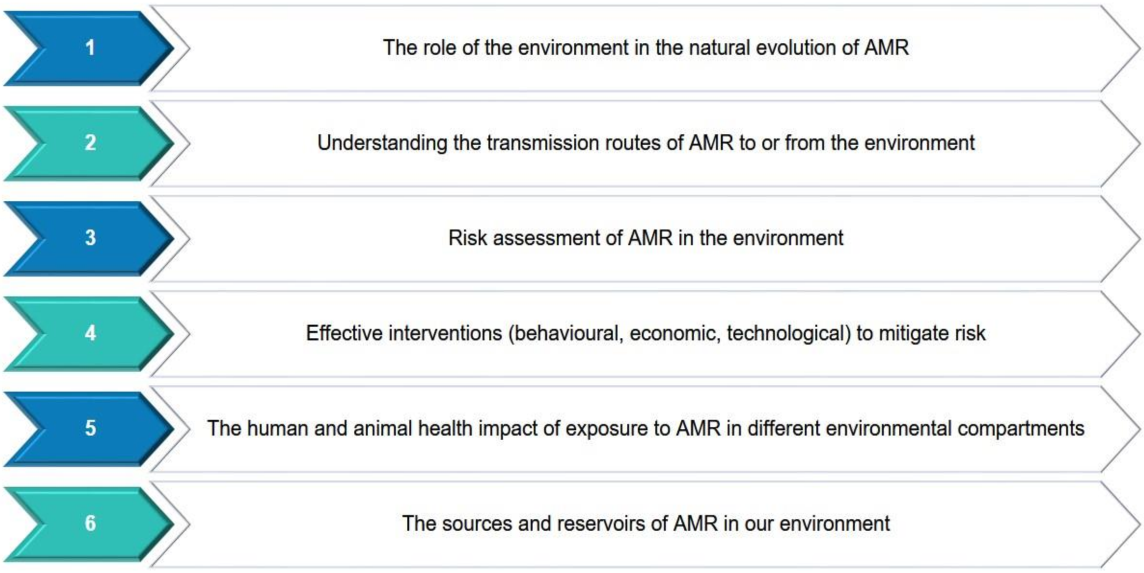


Figure 5.11. Ranking of key knowledge gaps relating to the environmental dimension of AMR, from least investigated (1) to most investigated (6).

Respondents were also asked to indicate additional areas of research they believe would add to our knowledge on AMR in the environment. Overall, each area was identified as an important area requiring further research by at least 40% of the survey respondents (Figure 5.12). Among all respondents, 82% (n=18) indicated the need for further work in harmonising environmental surveillance approaches for AMR. This was followed by 77.3% (n=17) of respondents identifying the impact of climate change on AMR in the environment as an important area requiring future research.

In addition, respondents indicated that more research needs to be carried out and advances and investments made in the field of genomics. Investigating the genomic context of genes in bacteria living in mixed communities in the environment and functional genomics of unknown AMR related genes carried on mobile elements were all listed as areas requiring further investigations. However, as was mentioned by respondents better sequencing technologies and infrastructures to aid analysis and interpretation of genomic data need to be put in place. The need for further research on the fate of antimicrobials in the environment, the amplification of AMR in the environment and also investigations into the environment as a source of novel antimicrobials were all highlighted as other key areas relating to AMR and the environment by survey respondents.

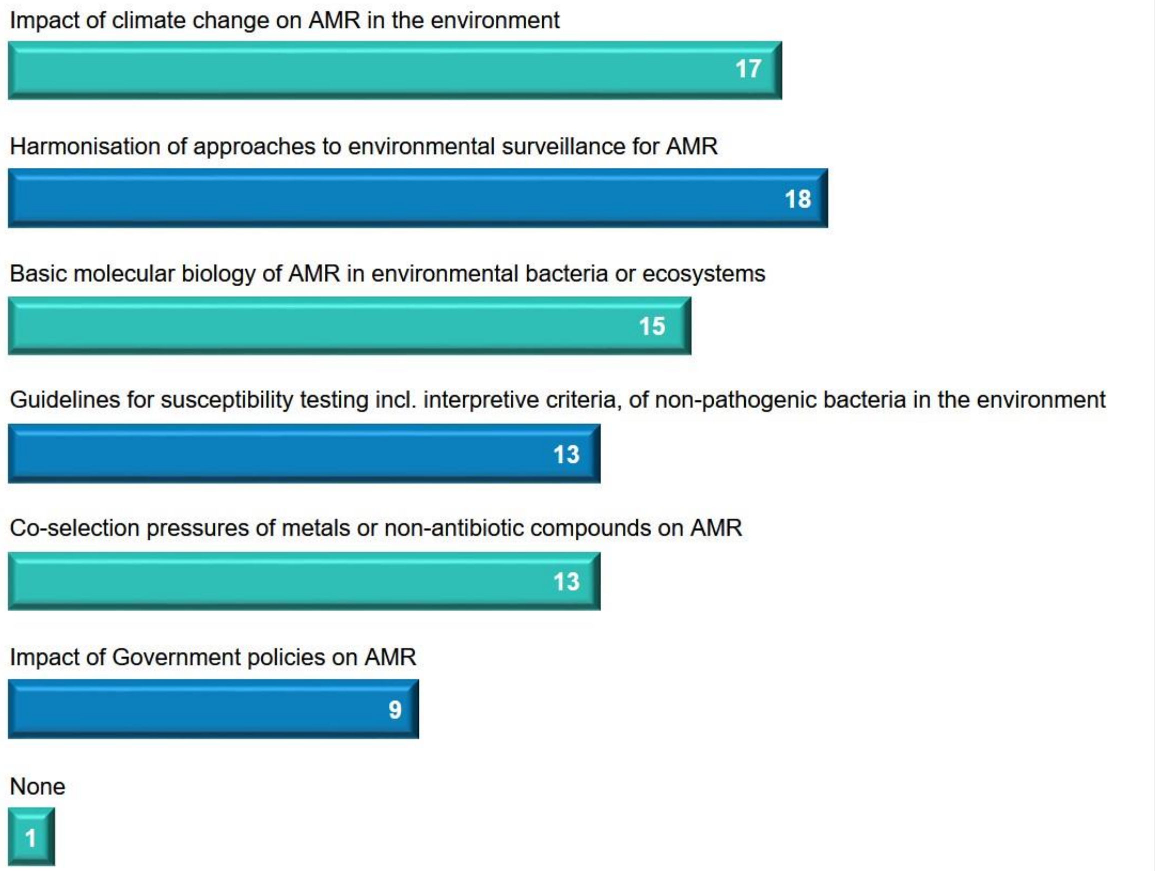


Figure 5.12. Number of respondents indicating the need for inclusion of these additional areas of research to enhance knowledge of AMR in the environment.

Overall, the poll and survey results provide an insight into the key knowledge gaps which currently exist in relation to the environmental dimension of AMR in the Irish context. Both the poll and survey results were used to plan and frame the discussion topics for the AMR and the environment stakeholder workshop outlined in Section 6.

6. Antimicrobial Resistance and the Environment Stakeholder Workshop

6.1 Workshop Overview

A virtual workshop was held with the aim to identify and inform research needs for the coming years in preparation of the second iteration of *i*NAP. The meeting took place on the 21st of April 2021 via an online based platform and was managed by Go West Conference and Event Management Ltd. This workshop was hosted by Prof. Dearbháile Morris (National University of Ireland Galway), Prof. Finola Leonard (University College Dublin) and Prof. Fiona Walsh (Maynooth University) and brought together key experts in the area of AMR and the environment and stakeholders in the human, animal and environmental health divisions in Ireland.

Overall, 38 key researchers and stakeholders were invited to attend. On the day of the event, 28 participants representing various research institutions, funding organisations and government bodies took part in the virtual workshop. Organisations represented included;

- Athlone Institute of Technology
- Department of Agriculture, Food and the Marine
- Department of Health
- Dublin City University
- Environmental Protection Agency
- Health Research Board
- Health Service Executive
- Limerick Institute of Technology
- Maynooth University
- National University of Ireland Galway
- Teagasc
- University College Cork
- University College Dublin
- University of Limerick

The workshop consisted of an introductory session, three breakout sessions and a closing session. The breakout sessions were chaired by Prof. Dearbháile Morris, Prof. Finola Leonard and Prof. Fiona Walsh with rapporteurs Dr. Louise O'Connor (National University of Ireland Galway), Ms. Niamh Cahill (National University of Ireland Galway) and Ms. Uchechi Okoroafor (Maynooth University). Throughout the breakout sessions the following topics, which were identified based on findings of the poll and survey outlined in Section 5, were discussed:

- Understanding the emergence and transmission routes of antimicrobial resistance to or from the environment

- The impact of exposure to antimicrobial resistance in different environmental compartments on human, animal and environmental health

- Reduction and risk assessment of antimicrobial resistance in the environment

6.2 Summary of Key Points Discussed and Knowledge Gaps Identified

Understanding the emergence and transmission routes of antimicrobial resistance to or from the environment

Potential sources of AMR in the environment in Ireland were discussed with participants highlighting those warranting further investigation as follows;

- Companion animals, in particular cats and dogs. It was suggested that future studies should investigate companion animals and their owners together due to the close contact between the two. If companion animals were identified as a source of concern, future studies would need to look at assessing the level of AMR contamination they contribute to areas such as beaches and walkways. In relation to dogs, the issue of dog fouling, which is a focus of concern at present from many perspectives including from a health perspective, was commented on during the workshop as something to consider as it could be a potential source of AMR into the environment.

- Wildlife, in particular deer, burrowing animals, bats and seagulls. In terms of wild deer, one participant indicated that preliminary results from a study currently being carried out in Ireland suggests that deer are a source of AMR, however, overall, it is area requiring further investigation.

In addition to deer, animals that burrow such as badgers and foxes were highlighted as a potential source currently under investigated in Ireland, particularly those in farmland areas or areas where manure or other waste has been spread. Although one participant mentioned that a study they had carried out previously did not identify badgers and foxes as major sources of AMR, they suggested that this may be an area worth revisiting and reinvestigating as there has been major advances in sequencing technologies since the study was completed. Wild rodents such as mice were also discussed as one participant spoke about poultry farms with inadequate biosecurity measures and mice transmitting salmonellae and suggested that mice may also have the potential to spread AMR from farms into the environment. Seagulls were also highlighted as a potential source of AMR in different environmental compartments, particularly as they feed typically on sewage waste piles and subsequently defecate across the environment. In addition to seagulls, bats were indicated as another potential source to consider as they too may carry AMR and people may come into contact unknowingly with their faeces in nature.

- Fish farms/aquaculture, particularly those where a lot of antibiotics are used.

- Effluent from abattoirs and knackeries as such waste will ultimately end up in the environment.

- Waste from pharmaceutical industries involved in the manufacture of antimicrobials as well as disinfectants may be potential sources or drivers of AMR in the environment.

- Graveyards/cemeteries, particularly those located in coastal locations or near water bodies, of which many would be historic cemeteries and therefore would not have been subjected to current planning laws.

The following potential key reservoirs and routes of transmission of AMR in the environment were highlighted by participants;

- Air, aerosols and dust particles - although there were varying views expressed as to whether these were of major concern or not, the fact that there has been very little work carried out, particularly in relation to long range transmission of dust particles and aerosols, indicates that there is a gap in this area in Ireland which may be worth investigating. The issue of climate change and the impact that may have on the dissemination of AMR was also commented on as well as the potential for intercountry and intercontinental transmission of dust particles under certain climatic conditions.

- Water bodies including groundwaters used for drinking water purposes, particularly those with septic tanks nearby, surface waters used for recreational activities, as well as land runoffs with which animals may come into contact. The potential importance of looking at materials such as microplastics to determine whether they are contributing to the persistence and transmission of AMR in water sources was also commented on.

- Soil - the importance of understanding the soil characteristics including structure and acidity, and soli usage such as tillage or pasture farming and the impact these factors may have on the persistence and/or transmission of AMR was commented on. In relation to climate change, the issue of wind and erosion and the impact that it may have on the transmission of AMR from soils into other environmental compartments including water bodies was highlighted.

- Sediments - climate change events such as flooding and storms were identified as important factors to take into consideration as such events could potentially churn up sediment and reintroduce AMR contamination into water sources. As indicated by one participant this may also be an important area to look at in terms of fish life and AMR transmission, particularly as these fish may subsequently be consumed by humans.

The issue of AMR amplification and co-selection due to the presence of antimicrobials or disinfection agents was also discussed among participants and highlighted as an area warranting further investigation. Participants felt it would be interesting to look in more depth at mobile genetic elements, plasmids and phage and how they are contributing to AMR, as well as metabolites and cocktails of metabolites and how they are contributing to selection within the environment. The issue of AMR amplification and co-selection is one of growing concern due to the COVID-19 pandemic, as increase in the use of both antimicrobials and disinfection agents has been noted. As such agents are ultimately being emitted into our wastewaters and subsequently into environmental water bodies, understanding the impact the release of such agents may have on levels of resistance in the environment and environmental biodiversity is important according to workshop participants.

The impact of exposure to antimicrobial resistance in different environmental compartments on human, animal and environmental health

In terms of AMR, the environment and its impact on animal and human health was considered among participants as a crucial step in the chain of transmission requiring further investigations. According to participants, once we have a better idea of key sources, reservoirs and transmission routes we will then be able to make progress in the investigation of the impacts of AMR exposure in these areas on human and animal health. In relation to the impact of exposure on human and animal health, following discussion among participants it was evident that there is a need for;

- Investigations into the mobilisation of ARGs from non-pathogenic environmental bacteria into human or animal pathogens. Identifying those most likely to be transmitted into human or animal pathogens and if possible, the potential impact this may have on human and animal health as well as the factors and mechanisms promoting this gene transfer were all indicated as areas requiring further investigation.

- The levels of resistant organisms or antimicrobials in the environment to be measured and determination of the maximum levels to which humans and animals can be exposed before promotion of resistance occurs. However, hazard characterisation is quite challenging, according to one participant, due to the difficulty in determining these levels to which one would need to be exposed before it becomes an issue, particularly as even if very low levels exist, this may still promote resistance.

- Both quantitative and qualitative assessments of AMR in the environment. One participant indicated the need for projects going forward to focus on both aspects as while it is extremely valuable, often there is a tendency to focus on quantitatively intense exposure to common and widely disseminated AMR, however, it is also important to investigate infrequent exposures to relatively rare or emerging AMR. This is important as exposure to rare or emerging AMR may compromise last line treatments and therefore have a more serious impact on human health.

- Further work to be carried out to identify and understand factors influencing acquisition, colonisation and infection in humans and animals following AMR exposure in the environment. In terms of human health, one participant highlighted that although AMR may not cause disease in the person to which it directly transmits, it may still have an impact as it begins to circulate in the human population and depending on the organism may later have an effect on morbidity or mortality. In addition to this another participant emphasised that exposure to AMR may not pose a risk to human health unless influenced by certain factors such as stress. In relation to animal health, another participant pointed out species and management system as factors which may influence the impact of exposure.

- The development of a harmonised approach for assessing the impact of AMR across different sectors.

- Investigations into the potential impact of septic tank leakages or overflows on contamination of pastures with AMR or antimicrobial residues, particularly as this may contribute to transmission of AMR or antimicrobial residues between humans and animals.

The impact of AMR on the natural biodiversity in the environment was also discussed throughout the breakout sessions, with participants highlighting the need for further investigations to;

- Better understand the presence, levels and fate of antimicrobials in the environment, as well as their impact on biodiversity. The maximum level of antimicrobials that we can allow into the environment before the natural biodiversity in the environment is impacted needs to be determined.

- Assess areas such as landfills and wastewaters for the presence of AMR or antimicrobials and the effect the release of such waste may have on the biodiversity in the receiving environment.

- Determine the impact of land spreading applications on biodiversity.

- Investigate bones and their environs, as antimicrobials have the ability to bind to bones, and evaluate the presence and impact of such antimicrobials in the environment.

- Investigate areas where other pollutants exist, such as heavy metals, which influence co-selection of ARGs.

- Assess the range of influencers promoting or driving resistance in an environment. With advances in genomic technologies, we are moving in the right direction, however this was identified as a challenging, complex and costly area to investigate.

Due to the lack of shared data among human, animal and environmental sectors, difficulties in bridging the gap between human and animal AMR infections and any associated role of the environment were emphasised. A discussion was had regarding the development of a centralised knowledge database in which human, animal and environmental sectors could work together by sharing information which could be hugely beneficial in assessing and monitoring AMR and associated infections in Ireland. The creation of such a network within Ireland could also allow us to collectively identify potential key factors contributing to AMR emergence, assess trends and predict outcomes across different locations which is of huge importance going forward. In addition, improving the collection of clinical data was thought to be critical to assessing the contribution of the environment to the problem of AMR in humans. As was highlighted during discussions, much more work needs to go into the harmonisation of data generation, analysis and overall digitalisation of data. Although moves have been made towards the use of GIS mapping, machine learning and fourth revolution technologies in assessing, monitoring and analysing contaminants of emerging concern, much more work needs to be done in the area of antimicrobials and AMR.

Reduction and risk assessment of antimicrobial resistance in the environment

The third topic discussed throughout the breakout sessions was 'Reduction and risk assessment of AMR in the environment', where the discussion was based around identifying ways to effectively assess risk and interventions to reduce risk of AMR in the environment.

In relation to risk assessment, the difficulty in identifying the model to use in measuring AMR risk in the environment was highlighted. There is a need to design and build a risk assessment model combining chemical, microbiological and modelling aspects in order to effectively assess AMR risk. At present, the method used to measure chemicals in the environment uses environmental quality standards and is based on measuring toxicity. In relation to developing a risk assessment model for AMR, the potential to modify the chemical model initially to risk assess for AMR was highlighted by one participant, while another suggested the use of a machine learning based approach. Potential challenges identified included how to actually measure the risk of AMR in the environment and associated costs, particularly due to the complexity of the environment and the many different environmental compartments. However, prior to developing a risk assessment model participants highlighted the need for;

- More research and longitudinal studies monitoring environmental compartments in order to gain a more in-depth understanding of changes in the environment over time and the factors such as climate change which may affect this. As pointed out during the discussions, there is difficulty in assessing the risk of AMR as we do not yet fully understand the impact.

- The type of data to be obtained to be determined, e.g. should data on pathogens or genes be employed as phenotypic and genotypic data can yield very different results. In addition to this, from a modelling aspect, a collection of data is required to parameterise, as well as to calibrate and then validate risk assessment methods.

- Further work around experimental design and the harmonisation and validation of methods around assessing levels of substances such as antimicrobial residues in the environment, along with the technologies and tools used. The importance of the generation and collection of good quality data which can be compared was emphasised.

- More work to be carried out in relation to the identification of ARGs, antimicrobial residues, metabolites and transformation products and understanding their mode of action. The importance of applying more advanced technologies or tools, such as long read sequencing through which we can both detect the pathogens as well as the AMR determinant, in the development of a risk assessment modal was commented on, however better infrastructure in Ireland is required to aid investigations and analysis.

In relation to the reduction of AMR in the environment, the importance of risk ranking was discussed and the potential this could have in identifying areas of perceived risk, allowing for interventions to be put in place. In terms of interventions, both behavioural and technological interventions were discussed. In relation to behavioural interventions, participants highlighted the need for;

- Better communication with the general public, health professionals and those working in the animal sector to educate people on the impact antimicrobials can have on the environment and subsequently on human or animal health and how to reduce the problem at source. The importance of encouraging health professionals to take into account levels of resistance in a community and the local environment and make better decisions around antimicrobial prescribing based on this was indicated.

- Further direction and strategy around coordinating and implementing better disposal and return schemes. Although there has been a move towards better antimicrobial disposal as a One Health disposal group was established with the aim to create a national system of collection of both human and animal antimicrobials, participants felt more could be done in this area to encourage better behaviour around the disposal of antimicrobials.

In relation to technological interventions for reducing the risk of AMR in the environment, although different methods for the treatment of human and animal waste exist, in order to determine whether modifications need to be carried out or further interventions put in place more work is required to;

- Assess the efficacy of existing treatments. In terms of wastewater management and treatment processes, there are uncertainties around the efficacy of AMR and antimicrobial residue removal / reduction. While in the agriculture sector there are concerns in relation to the potential presence of antimicrobial residues or AMR in manure despite treatment technologies such as anaerobic digestion and biogas production being employed in a small number of farms.

- Investigate the downstream effects of existing treatments and the effect they have on the environment. Although an intervention may benefit one area it may in fact create problems further afield such as in driving selective pressure. There were discussions around wastewater treatment and the impact the treated waste has on the receiving environment, particularly in relation to hospital effluent which was highlighted as an under-investigated area. There were also discussions around farm practices such as manure management and spreading and the potential impact such practices may have on the environment. One aspect of manure management identified included the emission of greenhouse gases and the potential effect or influence they may have on the resistome within samples. In addition to this, another participant spoke about hygiene practices and use of biocides as despite all of the benefits, these practices may drive AMR in the environment. It is important to ensure that these practices do not result in toxic or harmful wastes which may subsequently impact the environment.

Overall, it was indicated that assessing risk and determining ways to reduce the risk of AMR in the environment can prove quite challenging due to the diversity of environments. However, the idea of a multidisciplinary team working together, sharing expertise and focusing holistically and intensively on a perceived area of concern was suggested as a useful approach.

Antimicrobial design and development were highlighted as additional areas where improvements need to be made. As at present antimicrobial design is very much focused around efficacy in human or animal health, the need for improved design taking into consideration better degradation and effects on the environment was highlighted, with one participant commenting on the potential for the development of greener antimicrobials. The potential need for policy change and further research in this area was also highlighted throughout the discussions.

7. Conclusion and Recommendations

This report provides a comprehensive review of previous and ongoing research into the environmental dimension of AMR in the Irish context between 2015 and April 2021. This report also provides a detailed summary of findings arising from responses to the survey and discussions at the AMR and the Environment stakeholder workshop.

Research in Ireland to date has concentrated on identifying potential sources of AMR. There is evidence to suggest that human and animal wastes act as sources of multi-drug resistant organisms present in our environment. In addition, data suggest that biosolids generated during wastewater treatment processes and used as fertiliser on agricultural land may be a source of antimicrobials which may further select for resistant organisms in the environment. Studies examining the role of animal waste, including land-spreading of slurry, as a transmission route of ARBs from animals into the environment are underway. There is some evidence that suggests an association between livestock density and contamination of groundwaters with *E. coli* resistant to antimicrobials commonly used in veterinary medicine. In addition, contamination of groundwaters with anthelmintic and anticoccidial compounds was documented.

The aquatic environment (surface waters, ground waters, wastewaters) has been the environmental niche most studied to date. It is evident from the reviewed studies carried out in Ireland that both untreated and treated wastewaters, as well as surface and subsurface environments are potential sources and reservoirs of AMR and antimicrobial residues. However, these studies provide snap shots of data and are not comparable with the scale of AMR surveillance performed in animal and human studies in Ireland. Further studies of a more comprehensive nature using a harmonised approach across time and space are needed to build on these data.

Areas with few or no research in Ireland include soil and plants and the transfer of AMR from manure to these areas. Some current research is ongoing in this area but these are point prevalence studies rather than extensive analysis of the problem. Research is needed in these areas in order to generate baseline data for Ireland. Data on potential solutions as well as surveillance and sources are required in order to mitigate against the transfer and proliferation of AMR in these environments.

Recommendations for research priorities

High priority (Little to no knowledge available in Ireland)

- The impact of exposure to AMR and antimicrobials through aquatic environments on human and animal health is not well understood. Interventions to mitigate risk need to be explored. Understanding the factors that influence the colonisation and/or infection of humans and animals following exposure to AMR in the environment is key to development of risk management strategies.

- A nationwide analysis of AMR and antimicrobials in soil and plant environments across successive years is required to bridge the gaps in data present in these environments.

- Research into potential solutions to mitigate against the transfer of AMR and antimicrobials from animal and human sources to aquatic and terrestrial environments is required to stem the tide of AMR into the environment.

- Analysis of the roles of waste from sources not studied to date in the transmission, persistence and dissemination of AMR, for example, companion animals, wildlife, aquaculture, abattoirs, knackeries and pharmaceutical industries.

- Research into the role of the natural environment as a source and sink for ARGs and mobile genetic elements of human and animal health significance is key to understanding the environmental dimension of AMR. The anthropogenic impact on the biodiversity of natural environments receiving human and animal wastes which contain ARB, ARGs and antimicrobials is not well understood. Understanding the consequences for selection and persistence of AMR in our natural environment is key to development of risk management strategies.

- Analyse the impact of climate change on the dynamics of AMR in the environment.

- Analyse the importance of air as a route of AMR transmission.

Medium priority (Limited knowledge available in Ireland)

- Develop and implement standardised and rapid methodologies for sample collection, testing and reporting of antimicrobials and AMR in the environment. Adopting a standardised approach will facilitate data sharing and comparisons across the One Health paradigm, and support development of validated risk assessment tools.

- The role of the environment (waters, wastewaters, sediments) as a transmission and dissemination route for AMR warrants further study.

Low priority currently due to lack of baseline data to develop these aspects

- Examine and implement behavioural interventions through enhanced communication with the general public, health professionals and those working in the animal and environmental sectors.

- Develop and implement guidelines relating to the appropriate management and surveillance of wastewaters, effluents, and the receiving environments.

- Improve wastewater treatment processes to effectively reduce/prevent AMR and antimicrobial residues from entering the environment.

- Develop a centralised knowledge database in which human, animal and environmental data could be shared. Such a database would be hugely beneficial in assessing and monitoring in Ireland from a One Health perspective but is dependent on development of harmonised methodologies.

- Creation of risk assessment models of AMR transfer to the environment once there is sufficient data available.

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Abbreviations

AMR	Antimicrobial resistance
AMU	Antimicrobial use
ARB	Antimicrobial resistant bacteria
AREST	The Antimicrobial Resistance and the Environment Sources, persistence, Transmission and risk management project
ARGs	Antimicrobial resistant genes
CPE	Carbapenemase-producing *Enterobacterales*
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EPA	Environmental Protection Agency
ESBL	Extended spectrum β-lactamase
EU	European Union
FIB	Faecal indicator bacteria
GIS	Geographic information systems
HAC	High antibiotic consumption
HSE	Health Service Executive
***i*CRAG**	Irish Centre for Research in Applied Geosciences
***i*NAP**	Ireland's National Action Plan on Antimicrobial Resistance
JPI	Joint Programming Initiative
LAAs	Local authority areas
LAC	Low antibiotic consumption
LTCF	Long term care facilities
MGEs	Mobile genetic elements
NHWMP	National Hazardous Waste Management Plan
StARE	Stopping Antibiotic Resistance Evolution
TCC	Triclocarban
TCS	Triclosan
WWTP	Wastewater treatment plant

Appendix 1 Survey Distributed

Survey on Key Knowledge Gaps of Antimicrobial Resistance in the Environment

The purpose of this survey is to explore the knowledge gaps that exist with regard to the role of the environment in the persistence, transmission and evolution of antimicrobial resistance (AMR).

For the purposes of this survey, AMR refers to antimicrobial resistant organisms, antimicrobial resistance encoding genes and antimicrobial residues as appropriate.

The survey should take you no longer than 15 minutes to complete.

Please Note:

*When answering ranking questions, to ensure your answer will be submitted, please drag answers into position so that the number appears next to them.

*If you do not feel comfortable answering certain questions please leave blank and skip to the next question.

* Required

Consent to Participate

1. By confirming your consent below and submitting this form you are indicating that you understand the purpose of this survey, that you are participating voluntarily and that all information you submit can be collected.

   Please note - All surveys will be submitted anonymously. *

   ◯ I confirm my consent

AMR and the Environment

2. Have you been involved in research relating to AMR and the Environment in the last 5 years? If 'Yes' please proceed to Question 3 – If 'No' please proceed to Question 5.

   ◯ Yes

   ◯ No

3. If you have answered 'Yes' to Question 2, please list the areas you have worked on in the text box below.

4. If you have answered 'Yes' to Question 2, which organisation(s) did you receive funding from? Choose all that apply:

- [ ] Environmental Protection Agency
- [ ] Irish Research Council
- [ ] Science Foundation Ireland
- [ ] Health Research Board
- [ ] Marine Institute
- [ ] Department of Agriculture, Food and the Marine
- [ ] Safefood
- [ ] Geological Society Ireland
- [ ] Teagasc
- [ ] Enterprise Ireland
- [ ] Wellcome Trust
- [ ] EU Funding
- [ ] Other

5. With regard to key knowledge gaps in the environmental dimension of AMR, what do you think we know least about? Rank in order 1-6 (Least investigated = 1 Most investigated = 6)

The sources and reservoirs of AMR in our environment

The role of the environment in the natural evolution of AMR

Effective interventions (behavioural, economic, technological) to mitigate risk

Understanding the transmission routes of AMR to or from the environment

Risk assessment of AMR in the environment

The human and animal health impact of exposure to AMR in different environmental compartments

6. Do you think there are any other key knowledge gaps relating to AMR and the environment that have not been listed in Question 5? If Yes - please specify below. If No - please proceed to Question 7.

7. With regard to the sources of AMR in our environment, what waste sources do you think we know least about? Rank in order 1-7 (Least investigated = 1 Most investigated = 7)

Human

Companion animals

Livestock

Wildlife

Aquaculture

Industrial

Pharmaceutical

8. Are there any other sources of AMR you feel are under investigated that have not been listed in Question 7? If Yes - please specify below. If No - please proceed to Question 9.

9. With regard to environmental reservoirs of AMR, what do you think we know least about? Rank in order 1-6 (Least investigated = 1 Most investigated = 6)

Seawater

Freshwater (Surface water / Ground water)

Soil

Plants (edible for humans or animals)

Plants (non-edible for humans or animals)

Sediment (naturally occurring broken down material in water bodies etc.)

10. Are there any other environmental reservoirs of AMR you feel are under investigated that have not been listed in Question 9? If Yes - please specify below. If No - please proceed to Question 11.

11. Do you think the natural environment is the original source of many antimicrobial resistance genes in human and animal pathogens? If 'Yes' please proceed to Question 12 – If 'No' please proceed to Question 13.

◯ Yes

◯ No

12. If you have answered 'Yes' to Question 11, please specify below what areas you think need to be investigated in terms of understanding the role of the natural environment in the evolution of AMR of human and animal health importance?

13. With regard to effective interventions to mitigate the risk of AMR in the environment, what do you think we know least about? Rank in order 1-3 (Least investigated = 1 Most investigated = 3)

Behavioural Interventions (Social/Personal) (e.g. antimicrobial stewardship, personal responsibility/behaviours)

Economic Interventions (e.g. development and enforcement of policies and standards around appropriate antimicrobial use)

Technological Interventions (e.g. waste management and wastewater treatment systems)

14. Do you think there are any other effective interventions to mitigate the risk of AMR in the environment that are under investigated? If Yes - please specify below. If No - please proceed to Question 15.

15. With regard to the risk of transmission of AMR between the environment and humans, which transmission routes do you think we know least about? Rank in order 1-5 (Least investigated = 1 Most investigated = 5)

Transmission between air and humans

Transmission between seawater and humans

Transmission between freshwater (Ground water/ Surface water) and humans

Transmission between plants and humans

Transmission between soil and humans

16. With regard to the risk of transmission of AMR between the environment and animals, which transmission routes do you think we know least about? Rank in order 1-5 (Least investigated = 1 Most investigated = 5)

Transmission between air and animals

Transmission between seawater and animals

Transmission between freshwater (Ground water/ Surface water) and animals

Transmission between plants and animals

Transmission between soil and animals

17. With regard to the risk of transmission of AMR within the environment, which transmission routes do you think we know least about? Rank in order 1-10 (Least investigated = 1 Most investigated = 10)

Transmission between air and seawater

Transmission between air and freshwater (Ground water/ Surface water)

Transmission between air and plants

Transmission between air and soil

Transmission between seawater and freshwater (Ground water/ Surface water

Transmission between seawater and plants

Transmission between seawater and soil

Transmission between plants and freshwater (Ground water/ Surface water)

Transmission between plants and soil

Transmission between freshwater (Ground water/ Surface water) and soil

18. With regard to the risk of transmission of AMR between the environment, humans or animals, overall which transmission routes do you think we know least about? Rank in order 1-3 (Least investigated = 1 Most investigated = 3)

Transmission routes between humans and the environment

Transmission routes between animals and the environment

Transmission routes within the environment

19. With regard to the risk of transmission of AMR between the environment, humans or animals, are there any other transmission routes you feel are under investigated? If Yes - please specify below. If No - please proceed to Question 20.

20. With regard to the impact of exposure to AMR in different environmental compartments on human, animal and environmental health, what do you think we know least about? Rank in order 1-5 (Least investigated = 1 Most investigated = 5)

The risk of colonisation with antimicrobial resistant organisms for humans

The risk of colonisation with antimicrobial resistant organisms for animals

The risk of infection with antimicrobial resistant organisms for humans

The risk of infection with antimicrobial resistant organisms for animals

The risk of disruption to the natural biodiversity in the environment

21. With regard to the impact of exposure to AMR in different environmental compartments on human, animal and environmental health, is there anything else you feel is under investigated? If Yes - please specify below. If No - please proceed to Question 22.

22. Which of the following do you think should be included as important additional areas of research to add to our knowledge on AMR in the environment? Choose all that apply:

- ☐ Impact of climate change on AMR in the environment
- ☐ Harmonisation of approaches to environmental surveillance for AMR
- ☐ Basic molecular biology of AMR in environmental bacteria or ecosystems
- ☐ Guidelines for susceptibility testing incl. interpretive criteria, of non-pathogenic (humans or animals) bacteria in the environment
- ☐ Co-selection pressures of metals or non-antibiotic compounds on AMR
- ☐ Impact of Government policies on AMR
- ☐ None

23. If you have any comments or other key knowledge gaps relating to AMR and the environment please insert in the text box below.

Gap analysis of research needs to understand the environmental dimension of antimicrobial resistance in preparation for Ireland's second One Health Action Plan on Antimicrobial Resistance 2021-2025 (iNAP2)

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