

# Antimicrobial Resistance and the Environment – Sources, Persistence, Transmission and Risk Management (AREST)

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# Environmental Protection Agency

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2. Office of Environmental Enforcement
3. Office of Evidence and Assessment
4. Office of Radiation Protection and Environmental Monitoring
5. Office of Communications and Corporate Services

The EPA is assisted by advisory committees who meet regularly to discuss issues of concern and provide advice to the Board.

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## Identifying pressures

Antimicrobial resistance (AMR) is recognised globally as one of the greatest challenges to human and animal health. It has major implications for our agriculture and food production systems, the environment and the economy. The World Health Organization Global Action Plan on AMR sets out five key solutions to tackle the challenge of AMR. In 2021, Ireland's second National Action Plan on AMR (iNAP2) strengthened Ireland's commitment to tackling the challenge of AMR using the One Health approach, which recognises the link between human, animal and environmental health.

The role of the environment in the persistence and transmission of AMR is the least studied element of the One Health paradigm. This was further highlighted by the analysis commissioned by the EPA in preparation for iNAP2. The findings of the AREST project add significantly to our understanding of this environmental dimension of AMR.

## Informing policy

iNAP2 acknowledges that there remains a growing need to enhance our understanding of the environmental dimension of AMR and generate evidence to support the development and implementation of effective risk management and preventative strategies to halt further spread. The AREST project provides key data and recommendations to support iNAP2, in particular strategic objectives 2, 3 and 5, and informs several different national and international policies.<sup>1</sup>

## Developing solutions

The AREST project aligned with global and national One Health approaches in bringing together a wide range of partners in the areas of human health, animal health, agriculture, the environment, geographical information systems, risk assessment, high-throughput sequencing technologies and metagenomics. The AREST project (1) provides evidence of the extent of contamination of the Irish environment with antimicrobial-resistant organisms and antimicrobial resistance genes of clinical concern, (2) has generated national-level data on the key sources, hotspots and drivers of AMR in the environment from the human health and agriculture sectors, and (3) provides key data and recommendations to inform relevant policies and support Ireland's National Action Plan on AMR.

<sup>1</sup> Water Framework Directive (2000/60/EC); Bathing Water Directive (2006/7/EC); Groundwater Directive (2006/118/EC); EC (Drinking Water) Regulations 2014 (S.I. 122 of 2014); Environmental Impact Assessment Directive (85/337/EEC); Sewage Sludge Directive (86/278/EEC); Urban Waste-water Treatment Directive (91/271/EEC); Plant Protection Products Directive (91/414/EEC); Food Wise 2025; Food Vision 2030; National Farmed Animal Biosecurity Strategy 2021–2024; Working together for Animal Welfare: Ireland's Animal Welfare Strategy 2021–2025.

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This report is based on research carried out/data from 2018 to 2023. More recent data may have become available since the research was completed.

The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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# Executive Summary

Antimicrobial resistance (AMR) is recognised worldwide as one of the greatest threats to human health. Often referred to as the silent pandemic, AMR not only impacts on human health but has major implications for our animals, food production systems, environment and economy. The One Health concept recognises that the health of humans, animals and our environment are interlinked. Adopting a One Health approach is key to effectively tackling the global problem of AMR. The Irish government published Ireland's first National Action Plan on AMR (iNAP) in 2017. The publication of Ireland's second National Action Plan on AMR (iNAP2) in 2021 strengthens Ireland's commitment to taking a One Health approach to address the challenge of AMR. The role of the environment in the persistence and transmission of AMR is the part of the One Health paradigm that has not received adequate attention to date. Findings of the Antimicrobial Resistance and the Environment – Sources, Persistence, Transmission and Risk Management (AREST) project add significantly to our knowledge in this area.

The emergence and dissemination of AMR are related to the use of antimicrobial agents. Significant quantities of the antimicrobial agents administered to humans or animals are shed in urine and faeces. Humans and animals may have antimicrobial-resistant organisms (AROs) resident in their gut, which can enter the environment via their waste. The aquatic environment represents an important potential transmission route for AROs to humans, animals and the food chain.

Through the AREST project, a literature review of AMR in the environment revealed that natural aquatic environments are reservoirs of clinically significant antimicrobial resistance genes (ARGs). Mapping exercises identified appropriate "hotspots" and "cold spots" for sample collection through the point prevalence and longitudinal surveys of effluents and waters for the presence of AROs of clinical concern. The findings of these surveys provide evidence of the widespread contamination of Irish waters with AROs of clinical concern. The detection of AROs in water bodies classed as having good/excellent water

quality highlights limitations of current European Union bathing water quality criteria.

Many different types of AROs exist, including those that are resistant to our last-resort antimicrobials, e.g. carbapenemase-producing Enterobacterales (CPE). In many cases of infection with CPE, there are limited treatment options available, e.g. colistin. Unfortunately, increasing reports of plasmid-encoded colistin resistance (*mcr* genes) are emerging worldwide. Through the AREST project we provide evidence for the presence of CPE and *mcr* variants in the Irish environment.

The value of current treatment processes for human, animal and food wastes in reducing levels of AROs and ARGs has been disputed. Through the AREST project we have demonstrated that conventional manure treatment increases the relative abundances of grass ARGs and soil ARGs; however, current manure spreading practices reduce the likelihood of the spread of AMR to livestock. Data generated throughout this project revealed that integrated constructed wetlands greatly reduced AMR in effluent waters. Assessment of anaerobic digestion processes for removal of AROs and ARGs revealed that although anaerobic co-digestion could not eliminate all ARGs, there was a significant decrease in the survival time of some CPE.

Risk assessment methodologies were developed through the AREST project, to advance our understanding of the likelihood of AMR development arising from antimicrobial entry to Irish surface waters, as well as to evaluate the likelihood of risk to human health arising from ARO exposure in specific exposure scenarios. The findings revealed that relatively few published studies have examined Irish water bodies for antimicrobial residues, and that the healthcare sector is a larger potential contributor to AMR in the environment than the agriculture sector, with residues from the latter being more diffused in the environment. The project also revealed that users of recreational water bodies impacted by wastewater discharges are at an increased risk of illness associated with AROs; however, this risk can be lowered by the

implementation of mitigation measures such as wastewater treatment or physical modification of the bathing site.

Overall, the AREST project (1) provides evidence of the extent of contamination of the Irish environment with AROs and ARGs of clinical concern, (2) has

generated national-level data on the key sources, hotspots and drivers of AMR in the environment from the human health and agriculture sectors and (3) provides key data and recommendations to inform relevant policies and support Ireland's National Action Plan on AMR.

# 1 Introduction

## 1.1 Background

Antimicrobial resistance (AMR) is recognised as one of the top 10 greatest threats to human health, as acknowledged in a series of authoritative reports in Ireland and across the globe (WHO, 2015, 2019). It is estimated that by 2050, unless action is taken, 10 million deaths per year will be attributable to AMR (O'Neill, 2014). The One Health concept recognises the interconnection between human, animal and environmental health. Adopting a One Health approach is key to effectively tackling the problem of AMR, as acknowledged in the declaration of the G20 following its meeting in Rome in September 2021 (Seifman, 2021). The role of the environment in the persistence and transmission of AMR is the part of the One Health paradigm that has not received adequate attention to date, as recognised in key policy documents (European Commission, 2017). This was also recognised in an Environmental Protection Agency (EPA) publication outlining key knowledge gaps in the environmental dimension of AMR in Ireland (Cahill *et al.*, 2021). Ireland's second National Action Plan on AMR acknowledges that there remains a growing need to enhance our understanding of the environmental dimension of AMR and generate supportive evidence to develop and implement effective risk management and preventative strategies to halt further spread (Government of Ireland, 2021). The findings of the Antimicrobial Resistance and the Environment – Sources, Persistence, Transmission and Risk Management (AREST) project add significantly to our knowledge in this area, as outlined in this report.

While many different types of antimicrobial-resistant organisms (AROs) exist, those that are resistant to the last-resort antimicrobials, e.g. carbapenemase-producing Enterobacterales (CPE), are of most concern. In many cases of infection with CPE, the available treatment options are limited, e.g. to colistin, tigecycline and fosfomycin. Unfortunately, reports of resistance to these last-resort agents are emerging, including increasing reports of plasmid-encoded colistin resistance (mobile colistin resistance (*mcr*) genes). While *mcr* genes were initially detected in

pigs in China in 2015 (Liu *et al.*, 2016), they have since been detected in various human, animal and environmental sources across the globe (Elbediwi *et al.*, 2019). Indeed, as outlined in Chapter 4, through the AREST project we provide evidence for the presence of *mcr* variants in the Irish environment. The *mcr* story dramatically illustrates the links between humans, animals and the environment in the context of AMR and makes it imperative that a One Health approach is adopted to tackle the problem of AMR.

Although AMR is a naturally occurring phenomenon, the use of antimicrobial agents has caused significant increases in the emergence and dissemination of AMR. Recently, increasing attention has been given to the potential impact that discharges from humans and animals containing AROs, antimicrobial resistance genes (ARGs) and antimicrobials may have on the environment, and, subsequently, the pivotal role the environment may play in the dissemination of AMR to humans and animals.

A significant quantity of the antimicrobials administered to humans and animals is shed in urine or faeces in a form that is still biologically active. Furthermore, a high proportion of humans and animals may have AROs resident in their gut, significant numbers of which can ultimately enter the environment via the waste of that human or animal. In Ireland, as in most European countries, most human wastewater is treated in wastewater treatment plants (WWTPs) before discharge to the environment. Under the Urban Wastewater Treatment Regulations and European Union (EU) Water Framework Directive (WFD), the EPA monitors and reports annually on wastewater discharges. The most recent data from 2021 reveal that 12 of 174 large urban areas did not meet national and EU requirements for secondary wastewater treatment, and that untreated wastewater is being routinely discharged into rivers, estuaries and coastal waters at 32 locations around Ireland (EPA, 2022). Animal (agricultural, wild and companion) waste also represents a risk for transmission and persistence of AMR in soils and the aquatic environment. Increased productivity in the agricultural sector and increases in the national herds under Food Wise 2025 will

result in a significant increase in the waste produced by the agricultural sector (DAFM, 2015). However, under Food Vision 2030, the agri-food sector has committed to improving performances in this area and ensuring that growth of this economy is achieved in a sustainable manner (DAFM, 2021). In Ireland, as elsewhere in Europe, land spreading of organic wastes, including animal manures and wastewater sludge, is an important part of the circular economy. There is increasing concern that such practices may contribute to the presence of ARGs in resident soil bacteria and AROs in soil, and subsequently in the aquatic environment (Xie *et al.*, 2016; Van den Meersche *et al.*, 2020).

The aquatic environment represents an important potential transmission route for AMR to humans, animals and the food chain, as surface waters are often discharge points for wastewater or run-off from agricultural land, while also serving as sources of drinking water supplies and/or waters used for food production and recreational purposes. To date, the value of current treatment processes for human, animal and food wastes in reducing levels of AROs and ARGs has been disputed.

As detailed in this report, the AREST project has generated national-level data on the key sources, hotspots and drivers of AMR in the environment from various sectors (human, agriculture) using a combination of conventional microbiology, geographical information systems (GISs), high-throughput sequencing technologies and risk analyses. The evidence gathered in selected local authority areas (LAAs) representing good geographical separation and inclusion of key drivers has provided evidence of the extent of contamination of the environment with AROs and ARGs. In addition, various AMR mitigation strategies, including anaerobic digestion (AD) and integrated constructed wetlands (ICWs), were assessed throughout this project. Data generated will inform relevant policies to support Ireland's National Action Plan on AMR.

## **1.2 The AREST Project Aims**

The overall aims of the AREST project were to:

- generate national-level data on the key sources, hotspots and drivers of AMR in the environment from the human health and agriculture sectors;

- provide evidence of the extent of ARO contamination of the aquatic environment and assess the relative contribution of human healthcare, human wastewater and agriculture;
- embed the One Health concept and build the capacity of Ireland's research community to support Ireland's National Action Plan on AMR.

## **1.3 Report Structure**

This report provides an overview of the research conducted and outlines the key findings and recommendations. The research was carried out through five integrated work packages (WPs). Each chapter in this report provides an overview of the studies carried out in each WP and includes publications arising from this work to date.

The report is structured as follows:

- Chapter 2: review of the current state of knowledge on AMR in the environment.
- Chapter 3: mapping of key sources of AMR and identification of transmission routes into the environment.
- Chapter 4: assessment of the relative contributions of various sectors to AMR in the environment and examination of the role of various environmental reservoirs on persistence and transmission.
- Chapter 5: assessment of efficiencies of treatment processes for removal of AROs from wastewater and manure.
- Chapter 6: risk ranking of antimicrobial sources and ARO exposure assessment.
- Chapter 7: overall conclusions drawn from the AREST project and key recommendations for future work.

In line with Ireland's National Action Plan on Antimicrobial Resistance 2017–2020 (DoH, 2017), we use the term AMR throughout this report. In general, AMR refers to resistance to antibacterials (antibiotics), antivirals, antifungals and antiparasitics. For the purposes of this project and report, where we refer to AMR we are referring to resistance to antibacterials (antibiotics).

## 2 Systematic Literature Review of Antimicrobial Resistance in the Environment

### 2.1 Key Highlights

- Natural aquatic environments worldwide are reservoirs of, and dissemination routes for, clinically significant ARGs.
- A review of current literature revealed a lack of studies on pollution-free aquatic environments, thus highlighting the need for further investigations in these areas.
- Future work is required to develop a harmonised monitoring approach for environmental resistance surveillance.

### 2.2 Overview

A systematic review of all previous national and international studies and policy documents relating to AMR and the environment was carried out. This review involved identifying, collating and analysing data relating to the persistence and transmission routes of AMR in the natural aquatic environment. Owing to the level of interaction between humans and water bodies, e.g. through drinking and recreational activities, water bodies were chosen as the area of focus for this review, with a particular focus on those not in direct receipt of contaminating discharges. The overall aim of this review was to evaluate whether natural water bodies act as reservoirs for AMR, and to determine the significance of their role in AMR transmission. This review focused primarily on the clinically significant extended spectrum beta-lactamase (ESBL) and carbapenemase genes, and, in addition, examined the detection of mobile genetic elements (MGEs) in environmental isolates. Further details of the work outlined in this chapter, including the methods used and the findings and knowledge gaps identified, have been published elsewhere (Hooban *et al.*, 2020).

### 2.3 Methods and Materials

To direct and focus the scoping review, the following research question was defined: “What role does the natural aquatic environment play in the transmission of

ESBL and carbapenemase-encoding genes via mobile genetic elements?”.

Following the literature search, the screening of all articles retrieved and the elimination of articles based on pre-defined inclusion and exclusion criteria, 41 articles remained (see Hooban *et al.* (2020) for further details).

### 2.4 Key Findings and Discussion

The 41 studies analysed in this review derived from 19 different countries across five continents. Overall, 83% ( $n=34$ ) of the reviewed studies focused on inland waters, while the remaining 17% ( $n=7$ ) explored coastal/marine waters. The inland water systems investigated included lotic systems ( $n=29$ ), which are free-flowing waters, i.e. rivers ( $n=28$ ) and streams ( $n=1$ ); lentic systems ( $n=5$ ), which refer to still water bodies, i.e. lakes ( $n=4$ ) and ponds ( $n=1$ ); and subsurface/groundwater ( $n=2$ ) systems. The studies exploring coastal/marine waters investigated seawaters ( $n=6$ ) and bay waters ( $n=1$ ).

#### 2.4.1 *ESBL and carbapenemase genes in natural aquatic environments*

Overall, 80.5% ( $n=33$ ) of the studies reviewed reported ESBL and/or carbapenemase gene presence in aquatic environments across 16 different countries, demonstrating the widespread occurrence of these clinically relevant genes in natural aquatic environments worldwide.

ESBL genes were detected at a higher frequency than carbapenemase genes. In summary, ESBL genes were isolated in 20 out of 41 studies (48.8%), carbapenemase genes were recovered in four (9.8%), and nine (22%) studies detected the presence of both. This is most likely due to the circulation of ESBL genes for many years prior to the emergence of carbapenemases. The lower prevalence of these genes may also be attributable to lower usage of carbapenem antimicrobials, as in many countries



these antimicrobials remain a last-resort treatment option for multi-drug resistant (MDR) Gram-negative bacterial infections.

In summary, both ESBL and carbapenemase genes were detected in samples of groundwater, seawater, river water and lake water. In addition, ESBL genes were detected in bay water samples; however, there were no reports of carbapenemase gene presence in these samples. Overall, a wide variety of ESBL genes were reported, with the most prevalent being *bla*<sub>CTX-M</sub>, which was reported in 21 studies. Other variants included *bla*<sub>TEM</sub> (*n*=18), *bla*<sub>SHV</sub> (*n*=11), *bla*<sub>OXA</sub> (*n*=9), *bla*<sub>GES</sub> (*n*=1) and *bla*<sub>SFO</sub> (*n*=1). The most commonly detected carbapenemase gene was *bla*<sub>KPC</sub>, recovered in six studies, followed by *bla*<sub>NDM</sub> (*n*=3), *bla*<sub>VIM</sub> (*n*=3), *bla*<sub>IMP</sub> (*n*=2) and *bla*<sub>OXA</sub> (*n*=1). Some studies also reported the presence of multiple types of ESBL (*n*=20) or carbapenemase genes (*n*=2).

#### **2.4.2 Mobile genetic elements in natural aquatic environments**

A high proportion of the studies reviewed identified MGEs in various natural water bodies. In summary, MGEs were detected in 37 out of 41 (90.2%) studies carried out in 17 different countries. The most prevalent MGEs were integrons (*n*=22). These were predominantly class 1 integrons, which were recovered in 20 studies; class 2 integrons were recovered in six studies and class 3 integrons in one study. Other MGEs detected included plasmids (*n*=18), insertion elements (*n*=4) and transposons (*n*=3). The ability of environmental bacteria to successfully transfer ARGs via conjugation was also examined in 11 of the included studies. These findings highlight the potential for ARG dissemination among bacteria in

the aquatic environment. The presence of MGEs in uncontaminated aquatic environments may be a contributing factor to the presence of ARGs in such areas.

#### **2.4.3 Limitations and key knowledge gaps identified**

Hooban *et al.* (2020) outline the limitations of and critical knowledge gaps in current research identified in this review in further detail. However, their key observations included:

- When interpreting results, it is important to take into consideration publication bias: if negative or null results are withheld from publication this may potentially inflate the percentage of included studies with positive detections.
- There was a lack of standardisation among environmental sampling and processing methodologies across studies reviewed.
- The number of studies focusing on ARGs and AMR in uncontaminated aquatic environments was limited.

## **2.5 Conclusion**

In conclusion, the high proportion of studies identifying ESBL and/or carbapenemase genes, as well as MGEs, in various aquatic environments across the globe demonstrates the importance of these water bodies as reservoirs of and dissemination platforms for clinically significant ARGs. This review enabled the authors to identify key knowledge gaps in current research and pinpoint areas requiring further investigation, which subsequently informed the studies outlined in Chapters 3–6.

# 3 Mapping of Key Sources of Antimicrobial Resistance and Identification of Transmission Routes into the Environment

## 3.1 Key Highlights

- When combined with expert knowledge of AMR, GIS approaches are a useful tool to gain insights into the spatial dimension of AMR and to guide sampling campaigns.
- Mapping exercises enabled the identification of potential AMR hotspots, including sources and transmission routes, which were used to inform studies presented in Chapters 4 and 5.
- Potential future GIS applications may inform risk analysis and exposure assessment, as well as targeting of interventions.

## 3.2 Overview

The identification of potential AMR sources and transmission routes to the environment is important both to enhance understanding of AMR–environment dynamics and to inform and improve policy and surveillance systems. In this study, GIS approaches were used to identify potential key drivers, sources and transmission routes of AMR in the environment across four LAAs in the east, west and south of Ireland. The LAAs mapped were Galway city, County Galway, Fingal County and County Cork; they were selected based on geographical distribution, being representative of the national picture, and on the presence of potential sources of AMR in the environment. This chapter provides only an overview of the cartographic display generation, data layering and key findings from this study. Further details can be found in Chique *et al.* (2019).

## 3.3 Methods and Materials

### 3.3.1 Identification of variables and data selection for mapping

First, relevant variables to be mapped were identified and spatial datasets obtained. These were either obtained from Irish authorities (e.g. the EPA, the

Central Statistics Office, the Health Service Executive, the Health Protection Surveillance Centre) or independently produced through manual geolocation and geocoding, a process in which geographical coordinates are assigned to addresses for mapping purposes. Subsequently, a comprehensive review of relevant datasets was carried out and, following review, datasets were grouped into themes and categorised as sources or transmission routes. See Chique *et al.* (2019) for details on key datasets reviewed as part of this exercise.

### 3.3.2 Generation of cartographic displays and data layering

For each LAA, base maps, which comprised electoral districts (EDs) and small areas within the LAAs, were first generated using publicly available GIS boundary data. A weighted livestock “intensity” index was derived at the ED level based on estimated rates of antimicrobial use (AMU) in livestock categories and was used as a base map layer. The estimated rates of AMU in livestock categories were determined using available data on antibiotic sales in Europe (EMA and ESVAC, 2016) and the UK (VMD, 2015), in conjunction with expert knowledge. GIS approaches were then applied to extract, organise and collate the relevant selected data into final products in the form of thematic maps for visual and spatial analysis.

Various thematic maps at LAA level incorporating different data layer combinations were generated. On these, potential AMR sources and transmission routes were mapped to enable identification of different potential scenarios and pressures at landscape level relating to the spread of AMR. Through the GIS interface, additional data layers could be plotted to enable the user to evaluate the significance of different data variables and further assess AMR source clusters. Additionally, the GIS interface incorporates an interchangeable spatial scale function enabling data layers to be classified and viewed at different levels.

However, with data layering, despite interchangeable spatial resolutions, it is possible for graphics generated at LAA level to become overwhelmed with detail. As a result, cartographic displays adopting a finer spatial resolution were created. Fine-scale displays feature a higher level of data layer classification, enabling the user to include or exclude data based on their requirements. In addition, at this level, aerial imagery can be included to aid in the interpretation of landscape settings, identification of landscape features, e.g. farmland, and the planning of sampling campaigns.

### 3.4 Key Findings and Discussion

#### 3.4.1 Cartographic displays

Through this exercise, multiple cartographic displays were created. This section provides an overview of

some of the displays generated. Additional examples and further details relating to the displays created can be found in Chique *et al.* (2019).

Figure 3.1 illustrates potential AMR sources and livestock estimates identified in the LAAs County Cork (A), County Galway (B) and Fingal County (C). Overall, these displays highlighted “clusters” in each LAA at increased risk of harbouring AMR. Across all LAAs, AMR source clusters appeared to be predominantly located in urbanised areas, with County Cork the LAA with the most potential AMR sources.

Although these unclassified displays enable the identification of clusters, the information they provide is insufficient to further assess the individual sources in each cluster. By adding a higher level of detail to these displays, through source classification, it is possible to further assess these clusters. In this report, County Cork is used as an example. In Figure 3.1(A),

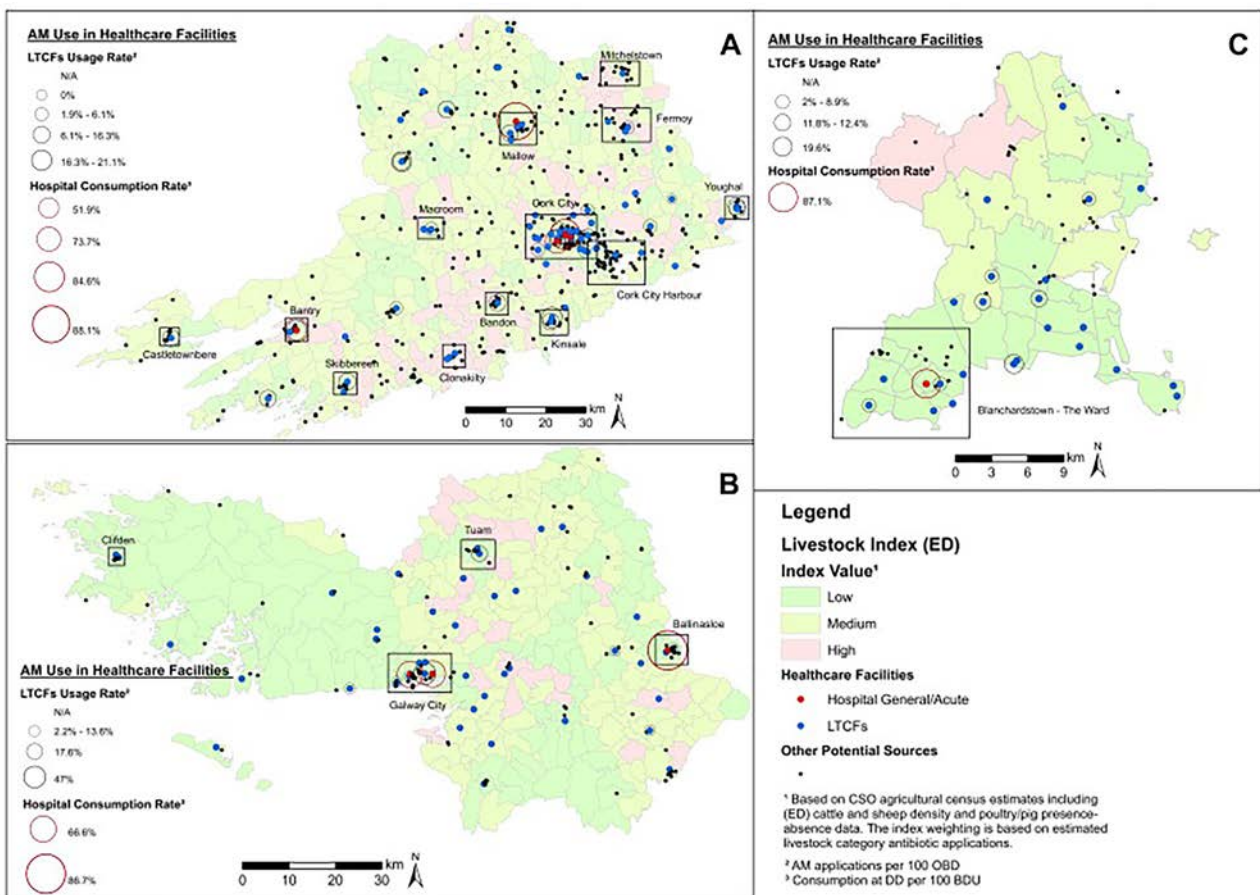


Figure 3.1. Composite map of potential sources of AMR (unclassified) and healthcare facilities (including antimicrobial (AM) use) in County Cork (A), County Galway (B) and Fingal County (C). Livestock index estimates are included as a base map layer. Clusters of AMR sources in urban areas are delineated. CSO, Central Statistics Office; LTCFs, long-term care facilities. Source: reproduced from Chique *et al.* (2019); licensed under CC BY 4.0 DEED (<https://creativecommons.org/licenses/by/4.0/>).

it is evident that there is an AMR source cluster within the County Cork ED Lehenagh. By providing a higher level of detail with regard to source classification, e.g. through the plotting of landfill sites, waste facilities, waste emission points, etc., it is clear that the majority of AMR sources in this particular area are waste emission points from a landfill site (Figure 3.2).

However, although cartographic displays at LAA level can provide valuable information in relation to the identification of AMR source clusters and potential sampling hotspots, they can, as a result of data congestion and poorer spatial resolution, become difficult to visualise and interpret. Through the generation of fine-scale displays, areas of interest identified in cartographic displays at LAA level could be assessed in greater detail. While many different fine-scale maps were created, one example generated

as part of this exercise is presented in Figure 3.3. This map illustrates potential sources of AMR, in addition to significant coastal/transitional water pressures (as indicated in the EPA–WFD datasets) present in the vicinity of Cork Harbour, i.e. urban wastewater, agriculture and anthropogenic pressures.

### 3.4.2 Data layering

Various cartographic displays were generated throughout this exercise, demonstrating different variables that can be combined to more accurately assess potential AMR sources and transmission routes and identify sampling hotspots. See Chique *et al.* (2019) for examples of the various key data layer combinations that can be used. Figure 3.4, illustrating County Cork, provides an example of how combining variables such as wastewater sources, drinking

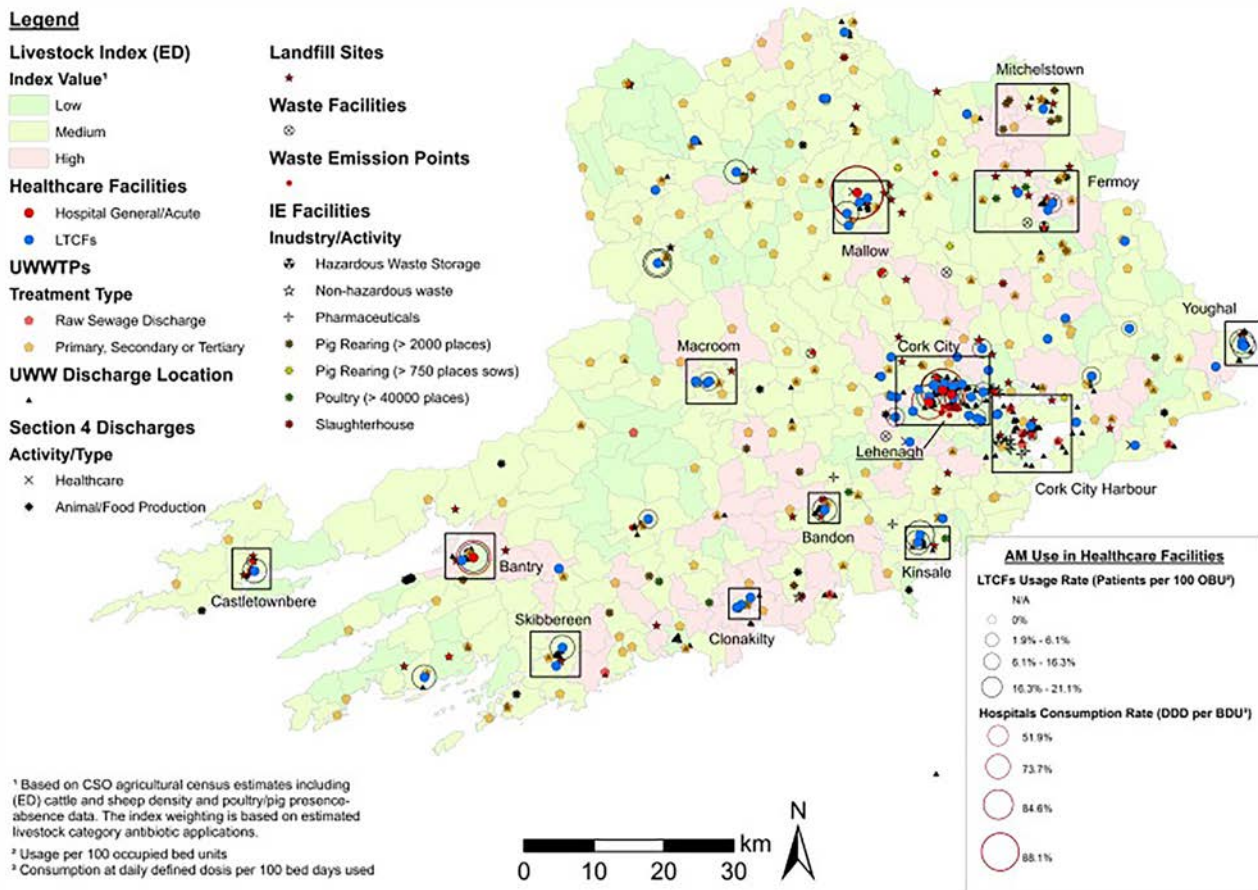
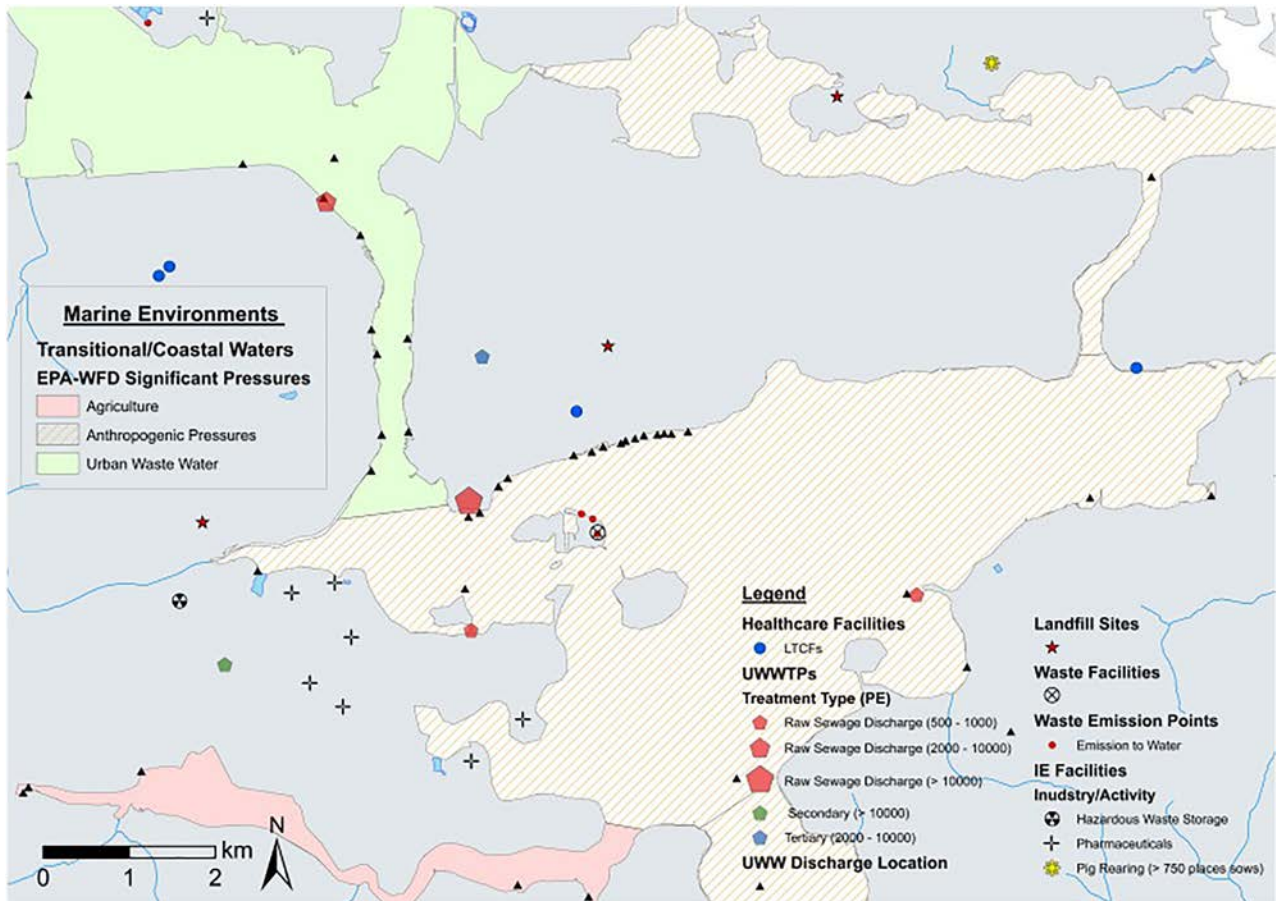


Figure 3.2. Map of potential sources of AMR (classified) and healthcare facilities (including AM use) in County Cork. Livestock index estimates are included as a base map layer. Clusters of AMR sources are delineated with the location of Lehenagh (underlined) – the ED with the largest number of AMR sources identified. IE, industrial emissions; OBU, occupied bed unit; UWW, urban wastewater; UWWTP, urban wastewater treatment plant. Source: reproduced from Chique *et al.* (2019); licensed under CC BY 4.0 DEED (<https://creativecommons.org/licenses/by/4.0/>).



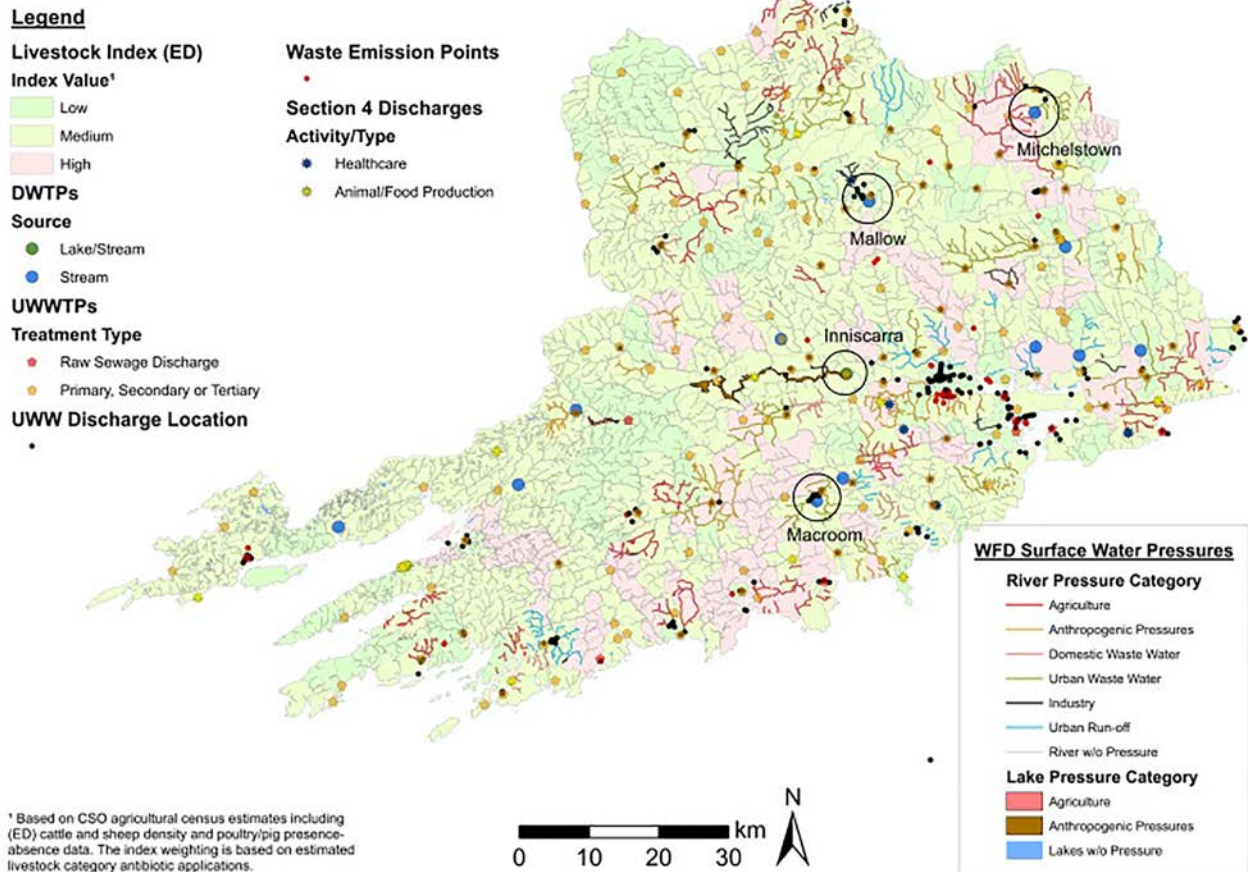
**Figure 3.3. Fine-scale, high-resolution map of potential sources of AMR (classified) and healthcare facilities in Cork Harbour, with significant coastal/transitional water pressures. EPA–WFD significant coastal/transitional water pressures data layers are included. IE, industrial emissions; PE, population equivalent (i.e. estimated population served by UWWTPs); UWW, urban wastewater; UWWTP, urban wastewater treatment plant. Source: reproduced from Chique *et al.* (2019); licensed under CC BY 4.0 DEED (<https://creativecommons.org/licenses/by/4.0/>).**

water treatment plants (DWTPs) and significant surface water pressures (EPA–WFD) can assist in the identification of drinking water supplies at risk of AMR contamination.

### 3.5 Conclusion

The findings of this exercise demonstrate how the implementation of GIS approaches can be used to identify potential AMR hotspots, such as sources and transmission routes, as well as water bodies

and supplies at risk of contamination, and therefore to subsequently guide sampling campaigns and determine intervention points. Overall, the findings highlighted the potential of using GIS, in addition to expert AMR knowledge, to enhance understanding of the spatial dimension of AMR and to evaluate different criteria, scenarios and pressures relating to the spread of AMR into the environment. The maps generated in this WP were used in other studies carried out as part of the AREST project to select suitable sampling hotspots (see Chapter 4).



**Figure 3.4. Map of drinking water treatment plants (DWTPs) and selected sources of AMR in County Cork. EPA–WFD significant river/lake pressures and livestock index estimates data layers are included. Relevant DWTPs are delineated with a buffer. UWW, urban wastewater; UWWTP, urban wastewater treatment plant. Source: reproduced from Chique *et al.* (2019); licensed under CC BY 4.0 DEED (<https://creativecommons.org/licenses/by/4.0/>).**

# 4 Assessment of the Relative Contributions of Various Sectors to Antimicrobial Resistance in the Environment and Examination of the Role of Various Environmental Reservoirs on Persistence and Transmission

## 4.1 Key Highlights

- Pollution, particularly from healthcare facilities, plays a significant role in the dissemination of antimicrobial-resistant bacteria (ARB) and ARGs to the aquatic environment.
- The presence of clinically significant ARB and ARGs in aquatic environments highlights the potential of these environments to act as AMR transmission routes to humans.
- The detection of clinically significant ARB and ARGs in water bodies classed as having good/excellent water quality highlights the limitations of current EU bathing water quality criteria.
- There is a need for regular monitoring of the aquatic environment for the presence of AMR both to better understand the complex role of environmental reservoirs in the selection, dissemination, transmission and persistence of AMR and to adequately inform policies to protect public health.

## 4.2 Overview

This section of the project aimed to assess the contributions of various sectors to AROs in the environment by:

- assessing and characterising the resistome of discharges from healthcare institutions, municipal wastewaters and agricultural waste, placing emphasis on resistances of critical importance to human health;
- examining the relative contribution of healthcare, municipal wastewater and agriculture to abundance and persistence of AROs in environmental waters and soils;
- examining the role of environmental reservoirs (waters and soil) in the transmission and persistence of AROs.

This chapter provides an overview of the tasks completed in this project, including point prevalence and longitudinal and metagenomic studies, and highlights the key findings observed. Data generated through this study were used to inform studies presented in Chapters 5 and 6.

## 4.3 Point Prevalence Study

### 4.3.1 Overview

A point prevalence survey was conducted initially to investigate the role that multiple different anthropogenic sources play in the dissemination of AMR to the natural aquatic environment on a national scale. In this study, there was a particular focus on the presence of CPE and ESBL-producing Enterobacterales (ESBL-PE). Further details of the methods used and of the findings can be found in Hooban *et al.* (2021).

### 4.3.2 Methods and materials

Overall, 39 water and 25 wastewater samples were collected between November 2018 and July 2019 across the four LAAs: Galway city, County Galway, County Cork and Fingal County. Suitable sampling locations were chosen based on maps generated by Chique *et al.* (2019) and on differences in population, land usage and bathing water quality. The sites included “hotspot” areas receiving wastewater discharges and “cold spot” areas receiving little to no contaminating discharges. Water bodies sampled included seawaters, rivers, lakes, estuaries and untreated water supplying DWTPs, while wastewaters included those from healthcare facilities (hospitals and nursing homes), airports and municipal WWTPs (including discharge of effluent from WWTPs that

receive wastewater from domestic households, commercial establishments and industry).

All water samples were filtered and assessed for total coliforms and total *Escherichia coli* using standard methods (Colilert-18). Both water and wastewater samples were then screened for the presence of CPE, ESBL-PE and fluoroquinolone-resistant Enterobacterales using phenotypic (i.e. culture-based and antimicrobial susceptibility testing (AST)) and genotypic methods (i.e. real-time polymerase chain reaction (PCR), whole-genome sequencing (WGS)).

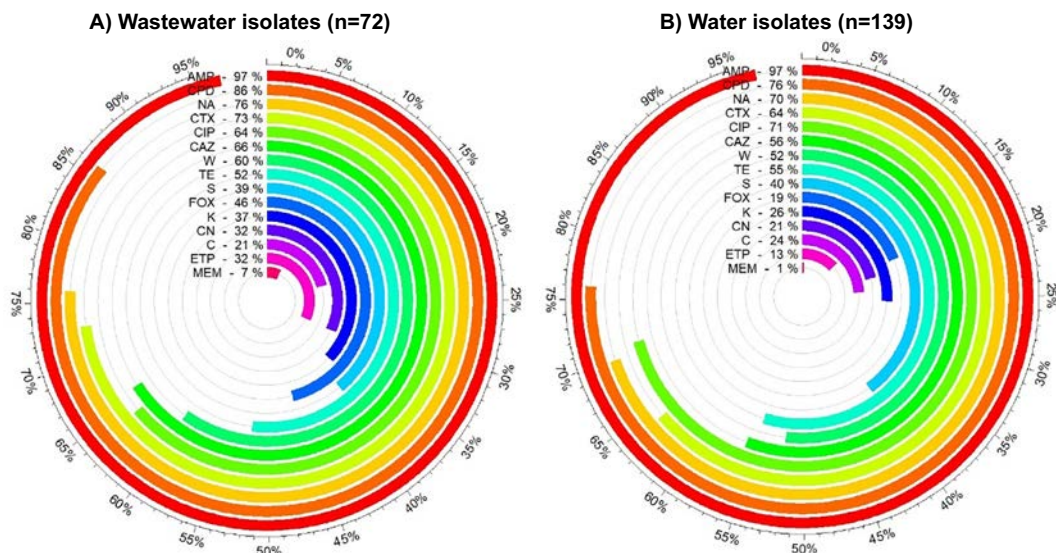
#### 4.3.3 Results and discussion

Based on the Colilert results, water bodies exhibiting the highest *E. coli* levels were seawaters and estuaries, while freshwaters displayed much lower levels. All samples exhibiting high levels of *E. coli* were collected in “hotspot” locations, with the exception of one estuarine sample. The presence of storm water overflows and a dense wildlife population were highlighted as potential contributing factors to higher *E. coli* levels in this “cold spot” location. Despite having higher levels of *E. coli* overall, based on the EU bathing water monitoring criteria (Directive 2006/7/EC), 83% ( $n=19$ ) of the seawater samples

tested ( $n=23$ ) were classed as having excellent water quality. However, detection of MDR bacteria, including ESBL-PE and CPE, in seawaters, in addition to wastewater and other aquatic environments throughout Ireland, highlights the limitations of the current EU bathing water monitoring criteria and of using *E. coli* as a sole indicator of water quality.

In summary, 211 Enterobacterales were isolated from water ( $n=139$ ) and wastewater ( $n=72$ ) samples. Through AST, high levels of resistance to some beta-lactam antimicrobials (ampicillin, cefpodoxime and cefotaxime) and to nalidixic acid and ciprofloxacin was observed among the isolates (Figure 4.1). Direct comparison of isolates revealed higher rates of resistance across wastewater isolates (10/15 (66.7%) of the antimicrobials tested) than across water isolates (Figure 4.1).

Real-time PCR confirmed the presence of ESBL-PE and CPE in multiple water and wastewater samples. In summary, out of 105 suspected ESBL-PE, 99 isolates (22 wastewater; 77 water) harboured a  $bla_{CTX-M}$  gene. With regard to carbapenem resistance, out of 42 isolates exhibiting resistance, 11 (6 wastewater; 5 water) were positive for one or more carbapenemase genes ( $bla_{OXA-48}$ ,  $bla_{KPC}$ ,  $bla_{NDM}$ ,  $bla_{IMP}$ ). While none of the samples from “cold spot” locations tested positive



**Figure 4.1.** Percentage of isolates displaying resistance to a panel of 15 antimicrobials in (A) wastewater and (B) water samples. AMP, ampicillin; CPD, cefpodoxime; NA, nalidixic acid; CTX, cefotaxime; CIP, ciprofloxacin; CAZ, ceftazidime; W, trimethoprim; TE, tetracycline; S, streptomycin; FOX, ceftoxitin; K, kanamycin; CN, gentamicin; C, chloramphenicol; ETP, ertapenem; MEM, meropenem. Source: reproduced from Hooban *et al.* (2021); licensed under CC BY-NC-ND 4.0 DEED (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).



for CPE, two out of five tested positive for ESBL-PE, revealing the circulation of clinically relevant ARB in environments with little to no known anthropogenic influence.

Based on these findings, a subset of 60 isolates (18 *Klebsiella*; 42 *E. coli*) were selected for WGS. Through the analysis, a wide range of ARGs were detected. Of particular concern were those that confer resistance to beta-lactams, including third-generation cephalosporins, and last-resort antimicrobials, carbapenems. Overall, the most prevalent beta-lactamase was *bla*<sub>CTX-M</sub> (*n*=48), followed by *bla*<sub>TEM-1</sub> (*n*=31), *bla*<sub>OXA-1</sub> (*n*=19) and *bla*<sub>SHV</sub> (*n*=16). Other ARGs identified in water and wastewater samples included those linked with resistance to aminoglycoside, sulfonamide, trimethoprim, tetracycline, phenicol, macrolide, fosfomycin, quinolone and fluoroquinolone antimicrobials. The potential for ARG transmission among environmental isolates was indicated by the identification of various different plasmid replicons, the most prevalent being IncFIB (*n*=45), IncFII (*n*=39) and IncFIA (*n*=29).

While WGS analysis revealed a wide array of sequence types (STs) across wastewater and water isolates, the most prevalent were *E. coli* ST131, ST38 and ST10; in the case of *Klebsiella* isolates, the only repeated STs were ST405 and ST11. Comparisons of core genome multi-locus sequence typing (MLST) loci revealed the persistence of certain strains in the aquatic environment. In one case, similarities were observed among three pairs of *E. coli* isolates recovered from different waters. The detection of one pair of *E. coli* isolates in waters not in direct contact with one another highlighted the potential for natural persistence in the aquatic environment; however, this is an area requiring further research. The nexus between wastewater and environmental dissemination of MDR pathogens was also established by the detection of identical *Klebsiella* isolates in hospital wastewater and two nearby waters.

#### 4.3.4 Conclusions

Overall, the results of this study indicated the widespread occurrence of clinically significant ARB and ARGs in wastewater and aquatic environments throughout Ireland. The need for further research on AMR in the environment is evident, with findings highlighting the importance of investigating both

“hotspot” and “cold spot” locations. Data generated through this study were used to identify optimal sampling locations for the longitudinal survey (section 4.4), which was carried out to further assess water and wastewaters across Ireland for AMR.

## 4.4 Longitudinal Study

### 4.4.1 Overview

As was evident from the point prevalence study conducted, the natural aquatic environment acts as a reservoir of ARB. The aim of this study was to further assess selected sites of interest for the presence of antimicrobial-resistant Enterobacterales, in particular ESBL-PE and CPE. Sampling sites were selected based on the results of the point prevalence study and, in addition to wastewaters, included waters receiving anthropogenic discharges as well as waters free from anthropogenic pollution. This section provides an overview of the work presented by Hooban *et al.* (2022).

### 4.4.2 Methods and materials

Samples of wastewater (*n*=36) and water (*n*=118) were collected on a seasonal basis across the same four LAAs investigated in the point prevalence study between July 2019 and November 2020. The types of water and wastewater samples collected in this study reflected those collected in the point prevalence study. All water samples were filtered and assessed for total coliforms and total *E. coli* using standard methods (Colilert-18). Phenotypic (i.e. culture-based and AST) methods were used to screen all water and wastewater samples for the presence of CPE, ESBL-PE and fluoroquinolone-resistant Enterobacterales. Subsequently, based on results, relevant members of the order Enterobacterales were further assessed using genotypic (i.e. real-time PCR, WGS) methods.

### 4.4.3 Results and discussion

Similarly to the point prevalence study, the findings of this study revealed the widespread presence of MDR bacteria in wastewater and aquatic environments throughout Ireland. AST on 419 Enterobacterales (348 water; 71 wastewater) revealed high rates of resistance to some beta-lactam antimicrobials

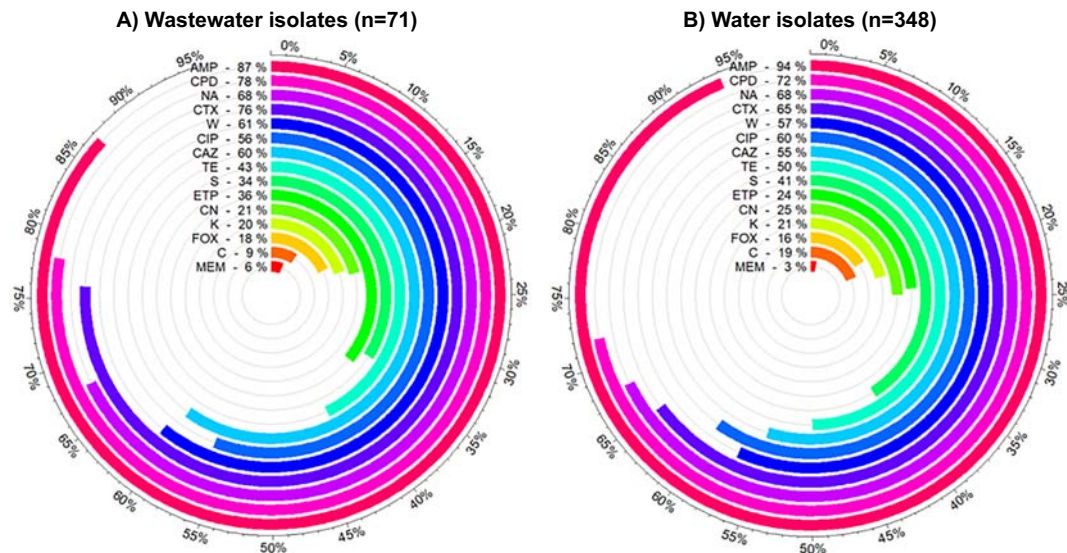
(ampicillin, cefpodoxime and cefotaxime) and nalidixic acid (Figure 4.2).

Widespread dissemination of ESBL producers was evident, as  $bla_{CTX-M}$  genes were detected in 92 (78%) water and 18 (50%) wastewater samples using real-time PCR. Using WGS analysis, other ESBL genes, including  $bla_{SHV-12}$ ,  $bla_{SHV-106}$  and  $bla_{OXA-17}$  were also identified. In addition to ESBL-PE, CPE were also detected in water ( $n=8$ ) and wastewater ( $n=5$ ) samples, as carbapenemase genes were identified using real-time PCR and WGS. Figure 4.3 displays the carbapenemase genes detected and sample types from which they were recovered. The most prevalent gene was  $bla_{OXA-48}$  ( $n=18$ ), followed by  $bla_{NDM}$  ( $n=14$ ),  $bla_{KPC}$  ( $n=4$ ) and  $bla_{OXA-484}$  ( $n=1$ ). Overall, river samples harboured the widest variety of carbapenemase genes (Figure 4.3). While no CPE were detected in the six “cold spot” locations sampled, similarly to the findings of the point prevalence study, two out of six “cold spot” locations tested positive for ESBL-PE. The presence of storm water overflows, wildlife, houses and boats was indicated as a potential contributing factor to the presence of ESBL-PE in such locations.

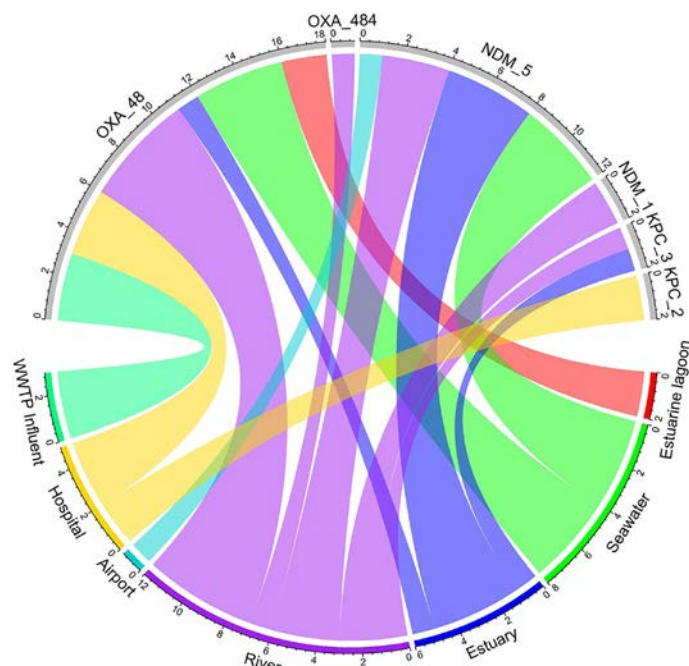
Analysis of the sequenced isolates ( $n=146$ ) revealed the presence of beta-lactamase genes in 138, with

the most prevalent being  $bla_{CTX-M-15}$ ,  $bla_{TEM-1B}$  and  $bla_{OXA-1}$ . In addition, a wide variety of other genes were identified, including those associated with conferring resistance to aminoglycoside, sulfonamide, trimethoprim, tetracycline, phenicol, macrolide, fosfomycin, quinolone, fluoroquinolone and colistin antimicrobials. See section 4.5 for further details relating to the *mcr* genes detected in these samples. The potential for widespread dissemination of ARGs was evident following the identification of at least one plasmid in the majority of the isolates (140/146) from water and wastewater samples. The most commonly detected was IncFIB ( $n=104$ ), followed by Col ( $n=96$ ) and IncFII ( $n=84$ ).

While a wide range of STs were noted among the sequenced *E. coli* and *Klebsiella* isolates from water and wastewater samples, the commonly detected included *E. coli* ST131, ST38 and ST10 and *Klebsiella* ST323, ST17, ST405 and ST309. Core genome MLST analysis on all sequenced isolates from the point prevalence and longitudinal studies revealed identical *E. coli* isolates and identical *Klebsiella* isolates in both wastewater and aquatic environments, highlighting the spread of these ARB to and within the aquatic environment.



**Figure 4.2.** Percentage of isolates displaying resistance to a panel of 15 antimicrobials in (A) wastewater and (B) water samples. AMP, ampicillin; CPD, cefpodoxime; NA, nalidixic acid; CTX, cefotaxime; CIP, ciprofloxacin; CAZ, ceftazidime; W, trimethoprim; TE, tetracycline; S, streptomycin; FOX, ceftoxitin; K, kanamycin; CN, gentamicin; C, chloramphenicol; ETP, ertapenem; MEM, meropenem. Source: reproduced from Hooban *et al.* (2022); licensed under CC BY 4.0 DEED (<https://creativecommons.org/licenses/by/4.0/>).



**Figure 4.3. Carbapenemase genes detected within Enterobacteriales isolated from different water and wastewater sources. Source: reproduced from Hooban *et al.* (2022); licensed under CC BY 4.0 DEED (<https://creativecommons.org/licenses/by/4.0/>).**

In addition to the Colilert results, when assessing water quality across seasons other potential factors affecting water quality (e.g. rainfall, distance to discharges and agricultural activity) were taken into consideration. While an increase in rainfall appeared to have an impact on coliform levels in natural waters, it was difficult to fully determine this impact, as, for most samples collected, only low to medium rainfall had been recorded in the days prior to sampling. In samples from areas that did experience heavy rainfall prior to collection (four samples in Galway city), increased levels of coliforms and *E. coli* were observed. The presence of several storm water overflows nearby was highlighted as a likely contributing factor.

With regard to CPE and ESBL-PE, there appeared to be more seasonal variation in the presence of CPE than in the presence of ESBL-PE. In summary, ESBL-PE were detected in most samples tested across all seasons; however, while there was consistent detection of CPE in some waters, in others detection varied throughout the year. Overall, the detection of ESBL-PE and CPE in natural waters across different seasons is concerning and is an area requiring further research, particularly CPE, as

research focusing on their detection in natural waters across different seasons has been limited to date.

#### 4.4.4 Conclusions

The detection of MDR bacteria in natural aquatic environments across Ireland is concerning, particularly detection of CPE. The carbapenemase genes detected reflect those commonly identified in clinical samples, therefore indicating that similar resistance types are circulating in both clinical and aquatic environments. Overall, the need for regular monitoring of the aquatic environment for the presence of ARB and ARGs is evident.

### 4.5 Detection of Mobile Colistin Resistance Genes in Environmental Isolates

#### 4.5.1 Overview

*mcr* genes pose a significant threat to the treatment of MDR infections, as colistin remains a last-resort treatment option. Further analysis of antimicrobial-resistant Enterobacteriales detected through the point prevalence (section 4.3), longitudinal (section 4.4) and

ICW (section 5.5) studies revealed the presence of *mcr-8* and *mcr-9* genes in six individual environmental samples. While there have been reports of these genes in human, animal and environmental sources worldwide, to the authors' knowledge this is the first time they have been detected in the Irish environment. This section provides an overview of the methodologies used and key findings observed. Further details can be found in Cahill *et al.* (2023).

#### 4.5.2 Methods and materials

Environmental water ( $n=157$ ) and wastewater ( $n=157$ ) samples collected between November 2018 and November 2020 were initially assessed for AMR, as outlined previously by Hooban *et al.* (2021, 2022) and Prendergast *et al.* (2022). Following detection of *mcr* genes, further testing and analysis of all *mcr*-positive isolates was carried out, including colistin susceptibility testing via broth microdilution, as well as long-read sequencing and analysis – see Cahill *et al.* (2023) for further details.

#### 4.5.3 Results and discussion

Overall, the findings provide evidence that *mcr* genes are circulating in the environment, as eight *mcr*-positive Enterobacterales were isolated from two freshwater ( $n=2$ ), three wastewater ( $n=5$ ) and one ICW influent ( $n=1$ ) samples. Although these genes were detected in only eight samples overall, as this study did not actively investigate for these genes or colistin resistance, their prevalence in the environment is believed to be much higher. In summary, one isolate harboured *mcr-8* (*Klebsiella pneumoniae*), while the remaining seven (one *Klebsiella michiganensis*, one *Raoultella ornithinolytica*, two *E. coli*, one *Enterobacter ludwigii* and two *Enterobacter hormaechei*) harboured *mcr-9* genes. MLST analysis on the *E. coli*, *Klebsiella* and *Enterobacter* isolates revealed a variety of different STs, including *E. coli* ST635 and ST10, *K. pneumoniae* ST111, *K. michiganensis* ST260 and *E. hormaechei* ST133.

Phenotypic testing showed that all eight isolates were MDR. While resistance to a range of different antimicrobials was observed across all eight isolates, four of the *mcr-9* positive isolates demonstrated resistance to the last-resort antimicrobial, ertapenem.

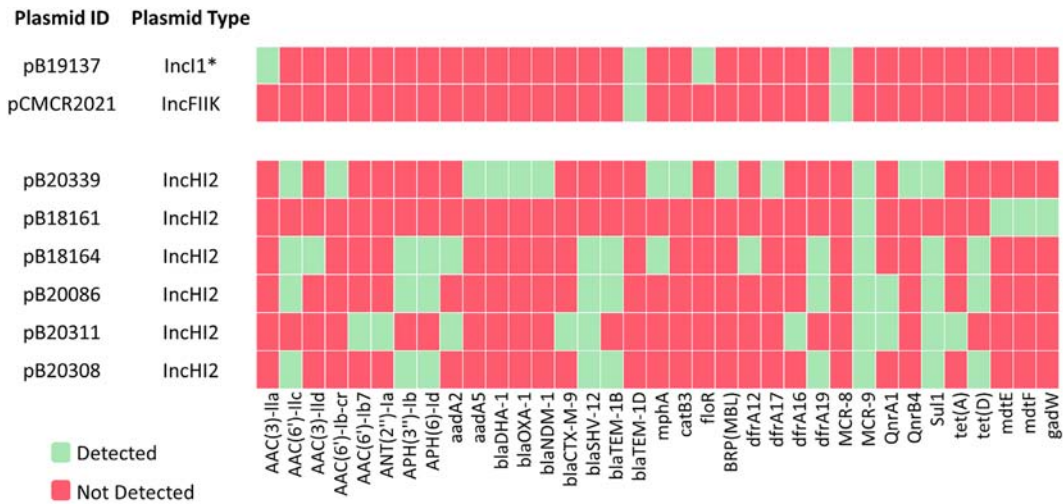
In relation to colistin, while the *mcr-8* positive *K. pneumoniae* demonstrated resistance, all *mcr-9*-harbouring Enterobacterales remained susceptible.

Although the *mcr-9*-harbouring isolates in this study did not show phenotypic resistance, their presence in the environment should not be overlooked, as, if the genes were expressed, colistin resistance may be induced.

Genotypic analysis revealed that these isolates harboured between 10 and 63 ARGs, including ESBL- (*bla*<sub>BEL-3</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-9</sub>, *bla*<sub>OXY-1-2</sub>, *bla*<sub>SHV-12</sub>) and carbapenemase- (*bla*<sub>OXA-48</sub>, *bla*<sub>NDM-1</sub>) encoding genes. The detection of fosfomycin resistance genes (*FosA*, *FosA2*, *FosA6*) was also concerning, as these genes have the potential to confer resistance to fosfomycin, which is generally reserved for the treatment of carbapenem-resistant infections. Of particular concern was the co-carriage of these genes on the same plasmids as the *mcr* genes, as this may significantly compromise treatment of MDR infections. Figure 4.4 shows the ARGs identified on the *mcr*-harbouring plasmids in this study.

The detection of all *mcr* genes on conjugative plasmids highlights their ability to mobilise among bacterial species and disseminate across different environments. While all *mcr-9* genes were located on IncHI2 plasmids, the *mcr-8* gene was detected on an IncI1-like plasmid. Insertion sequences were also found to flank the *mcr* genes in all plasmids, further facilitating ARG transfer.

In addition to the environmental isolates, one *mcr-8*-harbouring clinical isolate detected in 2021 by Ireland's National CPE Reference Laboratory was included in this study for comparison purposes, as, similarly to the environmental *mcr-8* isolate, this was identified as *K. pneumoniae* ST111. Based on core genome phylogeny, the isolates were found to be almost identical to one another, with an average nucleotide identity of 99.93% observed between both. Similarly to the environmental isolate, the clinical *mcr-8* isolate was phenotypically resistant to a range of different antimicrobial agents, including colistin, and harboured a range of ARGs, with an overlap of 18 ARGs observed between them. In addition, the clinical isolate displayed resistance to ertapenem and harboured the carbapenemase-encoding gene *bla*<sub>OXA-48</sub>.



**Figure 4.4. Antimicrobial resistance genes in the *mcr*-harbouring plasmids as determined by WGS analysis. \*Closest match determined by pMLST. Source: reproduced from Cahill et al. (2023); licensed under CC BY 4.0 DEED (<https://creativecommons.org/licenses/by/4.0/>).**

#### 4.5.4 Conclusions

The results highlight potential sources and reservoirs of *mcr* genes in the environment in Ireland. As inadequately treated wastewater, i.e. wastewater that has not been treated in compliance with EU standards, is still being discharged into the environment in some regions, the detection of *mcr* genes in wastewater samples is concerning. The discharge of such wastes may result in AMR contamination of surrounding aquatic environments, including those used for recreational or drinking water purposes. Overall, it is evident that there is a need for further investigations into the presence, persistence and dissemination of AMR, including *mcr*, in the environment.

## 4.6 Metagenomic Studies

### 4.6.1 Overview

This WP implemented a culture-independent approach in utilising deep shotgun metagenomics sequencing to assess the diversity, composition and function of microbial communities. This approach was adopted to provide insights into the microbial composition of anthropogenic-impacted aquatic ecosystems that were contaminated with AROs and ARGs. This analysis focused on a discharge point where communal untreated wastewater enters the seawater. Our results revealed rich and distinct microbial ecosystems between the different sampling locations. Our results also indicated that the discharge point seawater

microbiome was dominated by human enteric bacterial and archaeal species, including several opportunistic pathogens and AROs, which were subsequently transferred to the adjacent communal seawater. Overall, our results showed that the discharge point is a source of introduction of opportunistic pathogens and AROs in the seawater, with unchartered effects on local flora and fauna. The results also support the use of culture-independent methods as surveillance tools to assess the impact of anthropogenic contamination on affected ecosystems.

### 4.6.2 Methods and materials

A location in County Galway exhibiting anthropogenic contamination due to the introduction of untreated wastewater in the seawater was longitudinally sampled over five different dates and a period of 1 year. Four locations were sampled: (1) the discharge point, (2) seawater west of the discharge point, (3) seawater east of the discharge point and (4) an adjacent freshwater stream. To safely infer statistical significance three sampling replicates were collected each time. Following collection, 1 L of water was filtered through 0.45- $\mu$ m filter cups. The total environmental DNA was extracted from the filter and was processed for deep shotgun metagenomics sequencing (PE150, 30 Gb/sample). A complete metadata set was also collected, including water salinity, pH, temperature, wind, ultraviolet (UV) index, humidity, rainfall and tidal level. The raw reads

generated were filtered for quality and length, and bioinformatic analysis was conducted to assess the microbial diversity, composition and function of the samples. Alpha and beta diversity among the sampling points were evaluated using the Shannon and inverse Simpson metrics (a-diversity) and Bray–Curtis index (b-diversity). Owing to the high-dimensional nature of the results, statistical significance was assessed using Welch's *t*-test supplemented with the Benjamini–Hochberg procedure to control for the false discovery rate.

#### 4.6.3 Results and discussion

Over 1200 bacterial genera and 6000 bacterial species were identified across all sampling points, unravelling a highly rich and complex microbial ecosystem. Several archaeal and DNA viral species were also identified. Each sampling point presented with a distinct microbiome as assessed by alpha and beta diversity metrics. The bacteriome of the freshwater samples was dominated by bacterial genera associated with the soil and plant roots: *Bradyrhizobium* and *Streptomyces* (6.27% of the bacterial population). The seawater bacteriome was dominated by *Pelagibacter* and *Planktomarina* (3.41% of the bacterial population), which are associated with algae and play a key role in nitrogen fixation in the oceans. In contrast, the discharge point bacteriome was dominated by human enteric bacteria and opportunistic pathogens such as *Flavobacterium* and *Pseudomonas* (6.6% of the bacterial population). Bacterial populations with a key role in the aquatic ecosystem, such as *Pelagibacter*, were suppressed at the discharge point due to the introduction of

human enterobacteria, while the local microbiome also contained a large amount (12% of sequences) of methanogenic archaea associated with the human gastrointestinal tract. All six ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) pathogens associated with virulence and AMR were detected both at the discharge point and in the adjacent communal seawater, with *Pseudomonas aeruginosa* dominating the ESKAPE pathogen population. Bacterial genomes encoding for carbapenemases (*bla*<sub>NDM-1</sub>) belonging to *Acinetobacter* spp. were also identified at the discharge point. Our longitudinal analysis indicated an over 300% increase in the total *Vibrio* spp. population in the seawater when the UV index was the highest (*n*=4). Overall, our results indicate that several antimicrobial-resistant and opportunistic human pathogens are introduced to the aquatic ecosystem via the discharge of untreated wastewater. This introduction negatively impacts the native microbial communities and leads to contamination of adjacent communal seawater with bacteria with pathogenic potential.

#### 4.6.4 Conclusions

Shotgun metagenomics is a powerful analytical tool that could enable the surveillance of communal waters for anthropogenic contamination, provide valuable insights into the effects of anthropogenic activity on the aquatic ecosystem and act as an early warning system for the emergence and dissemination of pathogens within the community.

# 5 Assessment of Efficiencies of Treatment Processes for Removal of Antimicrobial-resistant Organisms from Wastewater and Manure

## 5.1 Key Highlights

- Land spreading of manure/slurry increased the relative abundances of grass ARGs and soil ARGs and MGEs transiently; however, current manure spreading practices reduce the likelihood of spread of AMR to livestock.
- The influent into ICWs from all farm types contained AROs and ARGs. This treatment system greatly reduced but did not eliminate AMR in effluent waters.
- Biowastes, such as food waste (FW), pig manure (PM), dewatered anaerobic sludge (ANS) and aerobic-activated sludge (AS), were found to be important reservoirs of ARGs, and, while none of the AD processes in this study could eliminate all ARGs present, anaerobic co-digestion (AcoD) could inactivate CPE in systems with particular concentrations of total solids (TSs) and total volatile fatty acids (TVFAs).

## 5.2 Overview

In recent years there has been an increased focus on the role of agriculture, and particularly livestock production, in the transmission and dissemination of AMR to the environment and humans. Animal manure is recognised as an important reservoir of AROs and ARGs. With increased productivity in the agricultural sector there may be a significant increase in the organic waste produced by the sector. AROs and ARGs in soil can enter the food chain via contaminated foods of animal origin, crops or groundwater, and may impact on human health. It is therefore critically important to understand how various agricultural practices may influence the flow of AROs and ARGs between different sectors. While there is an increased focus on the need for the development of more sustainable, circular-based production practices, there is a paucity of data on how the implementation of such practices may influence AMR in a One Health context.

In order to address these knowledge gaps, the objectives of this WP were to:

- determine what role land spreading of manures from different sources plays in the dissemination of AROs and ARGs;
- assess the impact of low-cost slurry treatments on the ARG profile in bovine slurry;
- examine the impact of ICWs on removal of AROs;
- examine the impact of AD on removal of AROs and ARGs.

These are summarised in Figure 5.1.

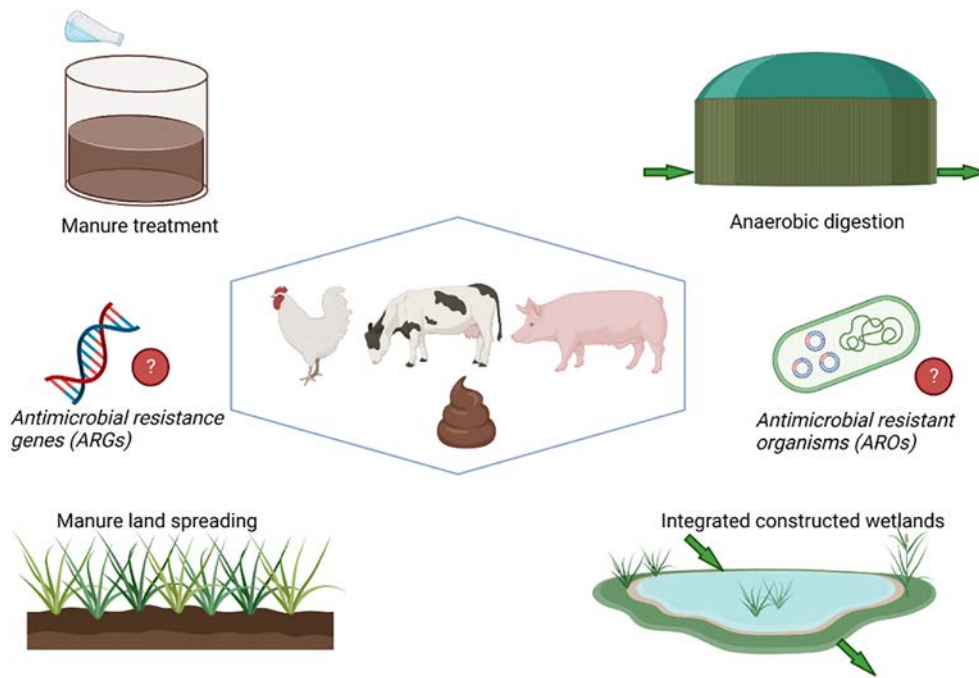
Three tasks were designed to address these objectives:

- Investigation of the role that manure/slurry from various agricultural sectors plays in the dissemination of AMR into grassland and soil, and the impact of manure treatments on the ARG profile in slurry.
- Assessment of the ability of ICWs to remove AROs from farm effluent.
- Evaluation of AD for removal of ARO from manure.

## 5.3 Impact of Land Spreading Manure on the Transmission of AROs

### 5.3.1 Overview

Land spreading of animal manures is an essential process in agriculture. Despite the importance of grassland in global food security, the potential of the grass phyllosphere as a reservoir of AMR is unclear (Tyrrell *et al.*, 2019). Additionally, the comparative risk of ARG dissemination associated with different manure sources is unclear. Because of the One Health nature of AMR, there is an urgent need to fully understand the transmission and dissemination routes of AMR at the agriculture–environmental nexus. We performed



**Figure 5.1. Agricultural practices examined by AREST that may influence AMR. Created with BioRender (<https://www.biorender.com>).**

a grassland field study to assess and compare the treatment and temporal impact of bovine, swine and poultry manure application on the grass and soil microbiome and resistome over a period of 4 months. Further details on the work presented here have been published by Tyrrell *et al.* (2023).

### 5.3.2 Methods and materials

Briefly, each of the manure types was applied to grass plots, with control plots also included, in a randomised block design. Soil and grass samples were collected from each plot on a number of occasions over the study period, and DNA was extracted from each. The samples were then analysed using 16S rRNA amplicon sequencing and high-throughput quantitative PCR (HT-qPCR). The soil and the grass phyllosphere contained a diverse range of ARGs and MGEs.

### 5.3.3 Results and discussion

- Manure treatment increased the relative abundances of grass ARGs and soil ARGs and MGEs, with increases in beta-lactam, aminoglycoside and sulfonamide resistance genes most frequently detected.

- Manure treatment resulted in the introduction of ARGs and MGEs, and temporal analysis of these genes indicated that ARG patterns were similar across the different manure types.
- Manure treatment resulted in both the enrichment in members of the indigenous microbiota and the introduction of manure-associated bacteria, with this impact extending past the recommended 6-week exclusion period. However, the relative abundance of these bacteria was low, and manure treatment was not found to significantly impact the overall composition of the microbiome or resistome.
- In both soil and grass samples, MGEs correlated with ARGs from clinically important antimicrobial classes, indicating the key role MGEs play in horizontal gene transfer (HGT) in agricultural grassland.

### 5.3.4 Conclusions

This study provided evidence that the current guidelines on withholding livestock from pasture after manure spreading reduces the biological hazard of ARG dissemination to livestock. These results also demonstrate the role of the grass phyllosphere as an under-studied sink of AMR.



## **5.4 Impact of Bovine Slurry Treatment on the Profile of ARGs**

### **5.4.1 Overview**

Bovine slurry represents a valuable source of nutrients and is commonly spread on agricultural land for this reason. As identified in the AREST project and elsewhere, it is a source of AROs, ARGs and MGEs, which can be transmitted to the land following application. Before application to the land, slurry is commonly stored on farms and is a source of emissions of greenhouse gases. Treatment of the slurry with different amendments during this time period is being looked at as an intervention point to address greenhouse gas emissions. However, it is not clear what impact these amendments may have on the properties of the slurry or on the microbial communities within the slurry, including the AROs, ARGs and MGEs present. This is important for assessing the potential of AMR dissemination in the environment and the resultant risk this may pose to the food chain.

### **5.4.2 Methods and materials**

Bovine slurry was treated with sulfuric acid, gypsum or biochar and stored for a period of 3 months. Untreated slurry was also stored under the same conditions as a control. Samples were taken periodically over the storage period. DNA was extracted from the slurry samples, and a HT-qPCR array was used to assess the temporal impact of these slurry amendments on the presence of a range ( $n = 119$ ) of ARGs, integrons and mobile elements in the stored slurry.

### **5.4.3 Results and discussion**

The relative abundances of the selected ARGs, MGEs and integrons to the 16S rRNA gene were used to reflect the resistome over time in the control and treated slurry samples. In total, 117 of the 119 genes tested for were detected in 70 samples. It should be noted that these genes were selected based on the detection of ARGs in bovine slurry samples, as described by Tyrrell *et al.* (2023). In general, the relative abundance of most ARGs decreased over time following each of the treatments. Some exceptions were observed, such as an increased relative abundance of *intl1*, *sul1* and *sul2* genes at the final time point of the untreated samples compared with the first time point. Statistical analysis

of the data is ongoing to identify other relevant changes.

### **5.4.4 Conclusions**

While this represents a preliminary study, initial analysis suggests that treatment of bovine slurry with various amendments does not impact on the number of ARGs that can be detected in the matrix during storage. The relative abundance of certain ARGs decreased during storage, regardless of treatment. Further research is required to determine how these changes relate to the microbial community composition.

## **5.5 Impact of Integrated Constructed Wetlands on the Transmission of AROs**

### **5.5.1 Overview**

An ICW is a system of cells or ponds that are planted with specific plants and act as natural wastewater treatment systems. The ability of ICWs to remove critically important AROs from farm wastewater was investigated as part of AREST. Further details on this work can be found in Prendergast *et al.* (2022, 2023).

### **5.5.2 Methods and materials**

Influent samples from the untreated farm waste and effluent samples taken at the end of the ICW system were collected monthly from four ICWs serving different farm types (suckler, dairy, dairy and poultry, and pig). Selective media were used to screen for the presence of carbapenem-resistant organisms, plasmid-mediated and AmpC beta-lactamase-producing organisms (ESBL/pAmpC) and fluoroquinolone-resistant organisms. Resistant organisms recovered were investigated using WGS to gain a better understanding of the dissemination of third-generation cephalosporin and fluoroquinolone resistance genes between isolates in the environment. AMR genes and point mutations were identified and compared with the phenotypic results for a better understanding of the mechanisms of resistance and resistance transmission.

### **5.5.3 Results and discussion**

A total of 82 AROs were obtained, with the majority being *E. coli* ( $n = 79$ ). Of these 82 AROs, 72 were

recovered from 48 influent samples and 10 from 48 effluent samples ( $p < 0.001$ ). There was a seasonal effect on recovery, with fewer AROs recovered during winter compared with other seasons ( $p < 0.05$ ). On the other hand, there were no significant differences in the recovery of AROs from different farms. Consistent with the use of media selective for AROs, most isolates from each of the farms were MDR (99%), with 65% resistant to seven or more antimicrobials. A high prevalence of tetracycline, trimethoprim/sulfamethoxazole and ampicillin resistance was detected in isolates from all four farms but there were differences in ESBL levels, with 63% of the isolates recovered from farm 4 (pig) being ESBLs, compared with 18%, 36% and 4.5% recovered from farms 1 (suckler), 2 (dairy) and 3 (dairy and poultry), respectively. No carbapenemase-producing organisms were isolated. A wide variety of cephalosporin and fluoroquinolone resistance genes (MGEs and chromosomal mutations) were identified that explained the phenotypic AMR patterns in most isolates. A total of 31 plasmid replicon types were identified among the 82 isolates, with a subset of them ( $n = 24$ ) identified in *E. coli* isolates. Five plasmid replicons were confined to a single *E. cloacae* isolate, and two were confined to the sole *K. pneumoniae* isolate. Virulence genes associated with functions including stress, survival, regulation, iron uptake secretion systems, invasion, adherence and toxin production were identified.

#### 5.5.4 Conclusions

Our results showed that, while ICWs were effective in reducing the levels of critically important AROs in farm wastewater on all four farm types, AROs can persist following this method of wastewater treatment. These AROs are equipped with plasmids that could transmit ARGs of clinical relevance to the environment, which could ultimately lead to the contamination of drinking water or irrigation water used in crops and pose a risk to human or animal health.

## 5.6 Impact of Anaerobic Digestion on the Presence of ARGs/AROs

### 5.6.1 Overview

AD is a natural process involving the conversion of organic feedstock by microorganisms into biogas and digestate in the absence of oxygen. This process may impact on the presence of AROs and ARGs, but

there is limited data in this regard. This study aimed to investigate the capacity of AcoD to inactivate CPE and reduce ARGs and MGEs in PM and FW. The work presented here has been published by Wang *et al.* (2020, 2021).

### 5.6.2 Methods and materials

First the profile of the antimicrobial resistome in typical biowastes was analysed, and this was followed by an investigation of the antimicrobial resistome in AD. The following targets were selected for the inactivation of CPE during mesophilic AcoD of PM and FW: KPC-3-producing *K. pneumoniae* (NCTC 13438), NDM-1-producing *K. pneumoniae* (NCTC 13443) and OXA-48-producing *K. pneumoniae* (NCTC 13442). Samples were collected during AD, and agar plate culturing, matrix-assisted laser desorption/ionisation–time of flight mass spectrometry and real-time PCR were used for CPE isolation and identification. For the fate of ARGs and MGEs, samples were collected from anaerobic digesters operating at 35°C and 55°C, and at different TS contents, and were analysed using HT-qPCR and/or metagenomic sequencing techniques. The effect of other parameters on CPE inactivation, including TVFA, total ammonia nitrogen, free ammonia nitrogen and pH, was also assessed.

### 5.6.3 Results and discussion

Through laboratory-based experiments, this study demonstrated that AcoD can effectively decrease the survival time of CPE from 9 to 2 days by increasing the TS contents from 5% to 20%. Statistical analysis revealed that TVFA was the significant factor in inactivating KPC-3- and OXA-48-producing *K. pneumoniae*. Although HGT might have occurred in the reactors, as some unexpected carbapenem-resistant bacteria were observed during the process, such as carbapenemase-producing *E. coli* (OXA-48 and NDM-1) and *Citrobacter freundii* (OXA-48), all suspected carbapenemase-producing organisms were eliminated within 11 days.

A total of 19 ARG types (317 ARG subtypes) were detected in FW, PM, ANS and AS via HT-qPCR. The total abundances of ARGs in different biowastes were in the following order: PM ( $4.03 \times 10^{-1}$  copy of ARG/copy of 16S rRNA gene) > AS ( $2.93 \times 10^{-1}$  copy of ARG/copy of 16S rRNA gene) > ANS ( $2.23 \times 10^{-1}$  copy of ARG/copy of 16S rRNA gene) > FW ( $1.90 \times 10^{-1}$

copy of ARG/copy of 16S rRNA gene). In particular, 27 different OXA genes were detected in FW, indicating that FW is an essential reservoir of carbapenem resistance genes, and it should be managed carefully to prevent the dissemination of OXA-type genes. The lower diversity and abundance of ARGs in ANS than in AS also suggests that AD can reduce the overall level of AMR.

Our further study showed that both mesophilic wet and dry AcoD can reduce the diversity and total abundance of ARGs and MGEs, and that mesophilic dry AcoD (TS $\geq$ 20%) of FW and PM promoted the reduction of ARGs and MGEs when compared with mesophilic wet AcoD. It is worth mentioning that the proportions of different AMR mechanisms remained more or less constant in wet AcoD throughout the entire digestion period, whereas they varied significantly during the dry AcoD. It was hypothesised that the fates of ARGs would be highly correlated to their resistance mechanisms, as most of the absent genes in this study conferred efflux pump and antimicrobial deactivation mechanisms. The high ammonia stress in dry AcoD led to the reduction of protential hosts of ARGs and MGEs, resulting in lower HGT frequency and ARG abundance.

Thermophilic AD at different TS contents did not show significant differences in reducing the diversity of ARGs, as there was little change in the number of ARG subtypes in all digesters; however, it did promote the reduction of MGEs. Compared with thermophilic wet AcoD, thermophilic dry AcoD was more unstable, as it had much higher concentrations of TVFAs and ammonia nitrogen. According to the results, a low TS content is recommended for thermophilic AD when system stability and methane yield are considered.

#### **5.6.4 Conclusions**

Biowastes such as FW, PM, ANS and AS were important reservoirs of ARGs. In particular, FW and wastewater sludge were hotspots of carbapenemase-encoding genes. Mesophilic AcoD at high TS content is recommended for reducing ARB and ARGs from FW and PM, while thermophilic AcoD at low TS content is recommended. Although none of the AD processes assessed in this study could eliminate all ARGs present, it was evident that such processes, with the appropriate TS and TVFA concentrations, can be effective in reducing CPE survival time.

# 6 Risk Ranking of Antimicrobial Sources and Antimicrobial-resistant Organism Exposure Assessment

## 6.1 Key Highlights

- Relatively few published studies have examined Irish water bodies for antimicrobial residues. Five antimicrobials, namely azithromycin, ciprofloxacin, clarithromycin, metronidazole and trimethoprim, have been measured in Irish surface water systems in concentrations predicted to select for resistance.
- The healthcare sector is a larger potential contributor to AMR in the environment than the agriculture sector, with residues from the latter being more diffused in the environment.
- Macrolide antimicrobials are predicted to be a high-risk group in terms of potential for AMR development.
- Within the macrolide group, the risk of resistance development for each antimicrobial is in the order of clarithromycin > azithromycin > erythromycin. There is also a potential risk of antimicrobial residues impacting non-target organisms such as cyanobacteria.
- Users of recreational water bodies impacted by wastewater discharges are at an increased risk of illness associated with AROs. This risk can be lowered via the implementation of mitigation measures such as wastewater treatment or physical modification of the bathing site.

## 6.2 Overview

This WP aimed to develop risk assessment methodologies to advance understanding of the likelihood of AMR development arising from antimicrobial entry to Irish surface waters, as well as evaluate the likelihood of risk to human health arising from ARO exposure in specific exposure scenarios.

The objectives of this task were to:

- develop a risk-ranking protocol to assess the relative contribution of various sectors in Ireland (health, agriculture) on the sources and levels of antimicrobials in the environment;

- implement risk assessment protocols to assess human exposure to AROs in water sources.

Four individual tasks were identified as part of the WP:

- conduct a critical literature review to identify relevant risk factors and capture the state-of-the-art understanding of antimicrobial pollution and ARB in Ireland and worldwide;
- create a database comprising current data on AMU in healthcare and agricultural settings, as well as data relevant to the fate and behaviour of antimicrobials, such as excretion level, persistence in environmental media and other risk factors identified in the literature review task;
- develop a novel risk-ranking approach to comparatively assess the potential contribution of various usage sectors to levels of antimicrobial residues in Irish surface water bodies and the likelihood of resistance development in environmental bacteria;
- evaluate the potential risk to human health from recreational water use at a designated study site. This task aimed to quantitatively assess the risk of illness from waterborne exposure to ARB.

## 6.3 Literature Review

The burden of AMR on the economy, healthcare services and quality of life has been well documented. In addition, several international organisations have flagged AMR as a significant threat to humanity and have suggested that the risks posed to human health will worsen in the coming years if current trends continue.

Antimicrobials are used in various settings, and antimicrobial residues have been shown to be released into the environment through their use in healthcare and agriculture. Throughout the literature, it is suggested that antimicrobial residues in surface waters can cause selective pressure that leads to developing resistance among environmental bacteria and other microorganisms.

This study compiled and detailed relevant information on AMU levels and trends in the Irish context, which are primarily stable. Pathways of antimicrobial pollution into ground- and surface waters primarily consist of excretion from humans and animals as a result of low bioavailability. The factors that impact the release level of an antimicrobial to the environment were identified, including usage level, metabolism of the antimicrobial within humans and livestock, and the half-life of the antimicrobial within the environment.

Finally, this study compiles reports of positive detections of both antimicrobial residues and ARB detected in Irish surface water bodies. In Ireland, five antimicrobials – trimethoprim, azithromycin, clarithromycin, ciprofloxacin and metronidazole – have been detected in concentrations predicted to select for resistance development. This study emphasises the need for increased and regular sampling of Irish water bodies for antimicrobial residues and ARB. Few studies have examined Irish water bodies for antimicrobial residues in particular, and more data on these residue levels would help inform policy and validate future risk assessment efforts. The applicability of different risk assessment strategies to examine resistance development is also examined, with Monte Carlo modelling found to be particularly appropriate.

The findings of this work have been published by Monahan *et al.* (2021). Further details on different risk assessment methods, including probabilistic modelling with Monte Carlo simulation, multi-media models and single-media models, and the advantages and disadvantages of each for analysing the risks posed by AMR, can be found in the above publication.

## 6.4 Risk Ranking – Healthcare and Agriculture

### 6.4.1 Overview

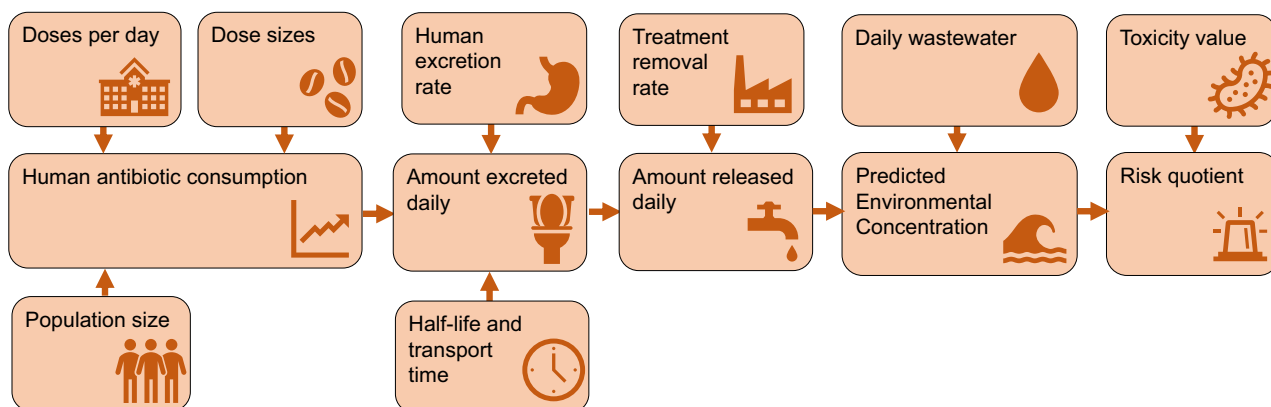
The presence of antimicrobial residues, from several sources, in surface water bodies can create selective pressure towards AMR development. Therefore, this study aimed to quantitatively evaluate the potential contribution of different AMU across sectors, specifically healthcare and agriculture, to AMR development in Irish surface waters.

### 6.4.2 Methods and materials

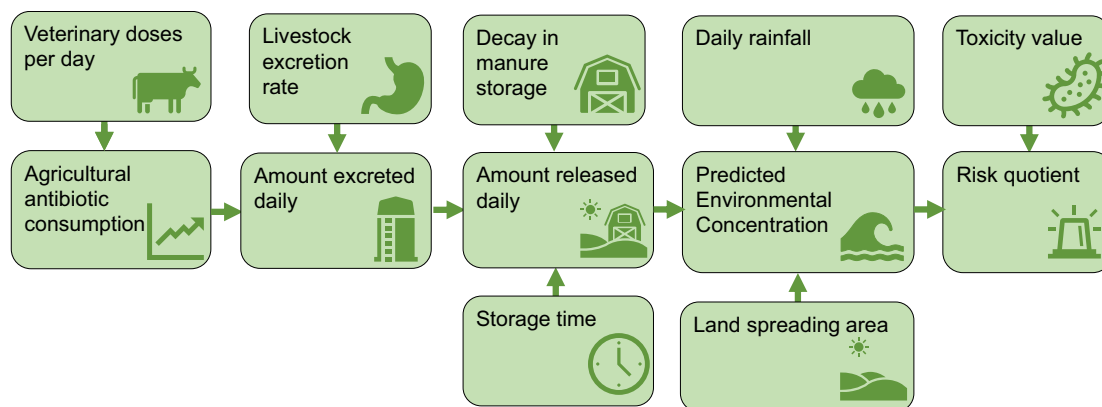
Paired probabilistic models were developed, one for each of healthcare and agriculture, in order to estimate likely run-off for each of six antimicrobial classes: penicillins, macrolides, tetracyclines, quinolones, sulfonamides and trimethoprim. Figures 6.1 and 6.2 demonstrate the structure of the models developed for healthcare and agriculture, respectively.

Predicted run-off was calculated via initial consideration of total antimicrobials consumed, in terms of grams per unit time, and via subsequent modelling stages simulating reduction of these levels before environmental release owing to factors such as metabolism, degradation and removal during wastewater treatment or manure storage. Published scientific literature was examined for data on these factors. These data were used to create probability distributions that best represented the input data.

Finally, dilution into receiving water bodies was simulated, providing a predicted environmental concentration (PEC) value. This PEC value was then



**Figure 6.1. Summarised framework for healthcare risk-ranking model of AMU.**



**Figure 6.2. Summarised framework for agricultural risk-ranking model of AMU.**

compared with a safety level, a predicted no-effect concentration, above which risk of resistance development is predicted. The ratio of these two values provides, for each antimicrobial class, a risk quotient (RQ) value. These RQ values are compared between sectors and classes to generate the final risk ranking.

#### 6.4.3 Results and conclusions

The model predictions were validated via comparison with previously detected concentrations of antimicrobial residues in Irish surface waters, with predicted levels being similar to previously detected ones. The average concentration of each antimicrobial class was also primarily in the same order in the predictions as in the sampling studies.

In terms of RQ, human-use antimicrobials were estimated as being of higher risk for resistance development than agricultural antimicrobials, with two “moderate-risk” and three “low-risk” classes, while each agricultural antimicrobial class was categorised as “no existing risk”. In particular, macrolides, with a mean RQ value of 3.8 for the class, were predicted to present the highest risk.

Potential avenues to reduce the environmental release of antimicrobials arising from each usage sector include, in the first instance, addressing them at source, and the use of more advanced wastewater treatment procedures, such as ozonation or constructed wetlands. This study also developed a novel model structure that can be applied to other regions to estimate or compare environmental release levels of antimicrobials.

The findings of this work have been published by Monahan *et al.* (2022), where further details on model development and risk assessment approaches can be found.

## 6.5 Priority Group Risk Ranking – Macrolides

### 6.5.1 Overview

A follow-up risk ranking was conceptualised, having identified human-use macrolides as particularly pertinent for AMR development in Irish surface water bodies. This model examined and compared three individual antimicrobials within the macrolide class – azithromycin, clarithromycin and erythromycin – as opposed to a class-wide analysis.

### 6.5.2 Methods and materials

A stepwise model from consumption to environmental release was constructed, and literature data were used to create probability distributions best representing input data. The stages modelled were administration, excretion, degradation in wastewater, removal rate during wastewater treatment, and dilution. This model also expanded on the previous study in several key ways:

- The impact of the route of administration on dose size, whether oral or parenteral, was considered as part of this model.
- Multiple wastewater treatment scenarios were examined to assess the potential change in run-off resulting from different treatment technologies.

Conventional activated sludge was assumed to represent the Irish scenario. In addition, an alternative membrane bioreactor treatment was modelled to illustrate the potential impact on release levels and risk.

- The risk level for ecotoxicity to other organisms, such as cyanobacteria and algae, was also examined, in addition to estimating risk levels for AMR development in surface waters.

### **6.5.3 Results and discussion**

Mean modelled RQ values of 0.51, 0.36 and 0.12 were found for clarithromycin, azithromycin and erythromycin, respectively, suggesting that clarithromycin was the antimicrobial with the highest risk of resistance development, followed by azithromycin and erythromycin. In contrast, azithromycin was estimated to pose a greater risk for ecotoxicity and potential disruption of ecosystem services, followed by clarithromycin and erythromycin, with mean RQ values of 4.31, 1.55 and 0.23, respectively. Results also highlighted the significant potential for membrane bioreactor technology to significantly arrest the environmental release of macrolide antimicrobials, with all three macrolides investigated having their final release levels reduced by at least 50%. The findings of this work have been published by Monahan *et al.* (2023), where further details on risk levels, potential impact and mitigation methods can be found.

## **6.6 Recreational Exposure Assessment**

### **6.6.1 Overview**

Having examined the likelihood of AMR arising in Irish surface waters, the next task focused on evaluating the likelihood of illness associated with AROs occurring as a result of recreational water use at designated study sites in Ireland. First, a designated site, a bathing zone in the west of Ireland potentially receiving ARB from two sources, a wastewater effluent run-off point and a freshwater stream draining agricultural land, was evaluated for risk of gastrointestinal illness arising from recreational water use.

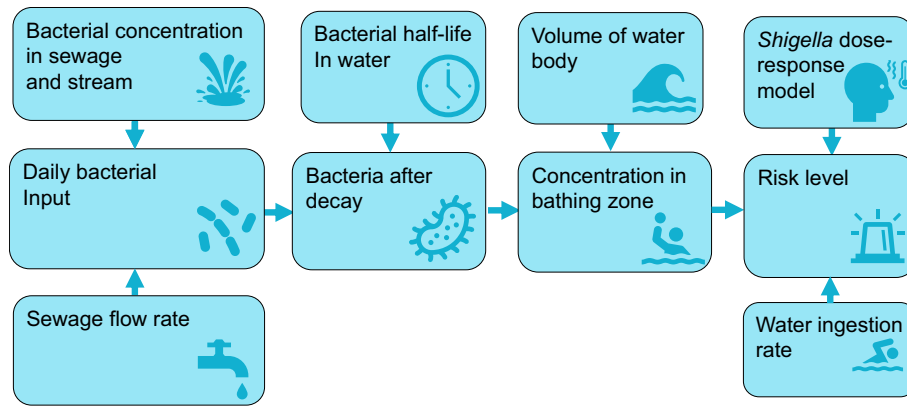
### **6.6.2 Methods and materials**

Sampling data, which quantified the concentration of *E. coli* resistant to ciprofloxacin, cefotaxime and ertapenem in the freshwater stream and wastewater outfall points near the bathing zone, were sourced from project partners, and probability distributions were fitted to the sampling data. A model was constructed to quantify the likely concentration of ARB at the bathing zone, with input data comprising flow rate data for ARB sources, sampling data on ARB concentration within the sources, decay rates for bacteria within the water column, and the physical parameters of the beach. Total numbers of ARB were calculated, and, after decay was simulated, numbers of surviving bacteria diluted into the body of water formed between the ARB point sources and the bathing zone were calculated. As the literature suggested that ARB detected within the freshwater stream may be present as a result of either agricultural use of antimicrobials or contamination during high tide resulting from the wastewater effluent run-off, two scenarios were considered. Scenario 1 assumed that both were independent inputs of ARB, and scenario 2 assumed that only the wastewater effluent outfall point was an ARB source. This approach is summarised in Figure 6.3.

A dose–response model was utilised to evaluate the likelihood of illness. Recent literature has suggested that the use of the dose–response model for *Shigella*, as a model for a highly infectious organism, is most appropriate for evaluating risk from ARB. Two dose–response scenarios were modelled, using both the *Shigella* model and the previous literature standard of using the dose–response model of antimicrobial-sensitive strains.

### **6.6.3 Results and discussion**

Predicted mean concentrations for ciprofloxacin, cefotaxime and ertapenem-resistant *E. coli* at the bathing zone were 0.75, –0.06 and –0.49 log CFU/L, respectively. In a worst-case scenario, the estimated risk of gastrointestinal illness to bathers was  $4.6 \times 10^{-4}$  per swim occasion for ciprofloxacin-resistant *E. coli*, with lower risk values for the other AROs.



**Figure 6.3. Summarised model structure for exposure assessment at recreational site.**

Implementation of wastewater treatment at the site and modification of the outfall site to increase the distance from the bathing zone could significantly reduce the likelihood of illness arising from the recreational use of this site. This study is the first to quantify the risk of illness associated with AROs arising from recreational water use in the Irish context.

## 6.7 Conclusions

The results of this body of work highlight the presence of antimicrobial residues and ARB in Irish waters. The presence of antimicrobial residues has been suggested to drive the development of ARB in surface waters via the creation of selective pressure. These ARB present in surface waters have the potential to negatively impact human health, causing illness and loss of life, as well as increasing pressure on healthcare services. The issue of ARB development

is a mounting threat to human well-being and requires action in terms of research, practice and legislation. Qualitative risk assessment methods are valuable tools in assessing the risk arising from different human health risks. Monte Carlo modelling is particularly good at accounting for uncertainty and variability in data on a given risk factor.

The findings of this work indicate that human-use antimicrobials, especially macrolides, are of particular concern for resistance development. Within the macrolide class, clarithromycin and azithromycin require vigilance for AMR development and ecological impact, respectively. This work strongly suggests that implementation of wastewater treatment has significant potential to reduce both antimicrobial entry into surface waters and human exposure to ARB. The outputs and results derived from this work can help drive and inform future efforts in antimicrobial stewardship, wastewater treatment and future research.



## 7 Recommendations

Overall, the AREST project aimed to increase knowledge of key sources, hotspots and drivers of AMR in the environment, and better understand the role the environment plays in the persistence and transmission of AMR to ultimately help protect natural resources, valuable ecosystems and public health.

This project adopted a One Health approach and brought together a wide range of experts in the areas of human health, animal health, agriculture, the environment, GIS, risk assessment, high-throughput sequencing technologies and metagenomics to achieve the project aims and objectives.

The following key recommendations are based on the findings and conclusions of the research studies presented in Chapters 2–6:

- Harmonisation of methodologies for environmental sampling and testing and reporting on antimicrobials and AMR in the environment is required to facilitate data sharing and enable data comparisons across the One Health paradigm.
  - There is a need for regular monitoring of AMR in aquatic environments. The potential for using shotgun metagenomics and GIS in the surveillance of aquatic environments for anthropogenic and AMR contamination was highlighted by the findings of this study. In addition, increased monitoring of the aquatic environment to quantify levels of antimicrobial residues is required to assist risk assessment efforts.
  - Limitations in current guidelines and criteria relating to water quality assessment were evident.
- Both international and national guidelines relating to the assessment of water quality need to take AMR into account. The development of guidelines for the appropriate surveillance and reporting of AMR and antimicrobial presence in the aquatic environment is recommended.
- Current wastewater treatment processes, both natural and conventional, are not designed to remove or reduce AROs, ARGs and/or antimicrobial residues from human and animal sources prior to discharge into the environment. Further research into potential solutions to effectively reduce/prevent entry of AMR through wastewater discharges into the natural environment is required.
  - Further investigations into AMR and antimicrobials in soil and grass environments is required to better assess the role of these environments in the persistence and transmission of AMR.
  - Development of dose–response models specific to ARB would be of benefit in assessing risks to human health arising from ARB as opposed to assuming dose–response models similar to those used for antimicrobial-sensitive organisms, or other model organisms.
  - Research into the impact of climatic conditions on various risk factors examined in this research, such as persistence half-lives, efficacy of wastewater treatment and bacterial decay within the water column, would be of benefit in reducing uncertainty as to the behaviour of antimicrobial residues and AROs in the environment.

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# Abbreviations

<b>AcoD</b>	Anaerobic co-digestion
<b>AD</b>	Anaerobic digestion
<b>AMR</b>	Antimicrobial resistance
<b>AMU</b>	Antimicrobial use
<b>ANS</b>	Anaerobic sludge
<b>ARB</b>	Antimicrobial-resistant bacteria
<b>AREST</b>	Antimicrobial Resistance and the Environment – Sources, Persistence, Transmission and Risk Management
<b>ARG</b>	Antimicrobial resistance gene
<b>ARO</b>	Antimicrobial-resistant organism
<b>AS</b>	Aerobic-activated sludge
<b>AST</b>	Antimicrobial susceptibility testing
<b>CPE</b>	Carbapenemase-producing Enterobacterales
<b>DWTP</b>	Drinking water treatment plant
<b>ED</b>	Electoral district
<b>EPA</b>	Environmental Protection Agency
<b>ESBL</b>	Extended spectrum beta-lactamase
<b>ESBL-PE</b>	Extended spectrum beta-lactamase-producing Enterobacterales
<b>ESKAPE</b>	<i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> and <i>Enterobacter</i>
<b>EU</b>	European Union
<b>FW</b>	Food waste
<b>GIS</b>	Geographical information system
<b>HGT</b>	Horizontal gene transfer
<b>HT-qPCR</b>	High-throughput quantitative PCR
<b>ICW</b>	Integrated constructed wetland
<b>LAA</b>	Local authority area
<b>mcr</b>	Mobile colistin resistance
<b>MDR</b>	Multi-drug resistant
<b>MGE</b>	Mobile genetic element
<b>MLST</b>	Multi-locus sequence typing
<b>PCR</b>	Polymerase chain reaction
<b>PEC</b>	Predicted environmental concentration
<b>PM</b>	Pig manure
<b>RQ</b>	Risk quotient
<b>rRNA</b>	Ribosomal ribonucleic acid
<b>ST</b>	Sequence type
<b>TS</b>	Total solid
<b>TVFA</b>	Total volatile fatty acid
<b>UV</b>	Ultraviolet
<b>WFD</b>	Water Framework Directive
<b>WGS</b>	Whole-genome sequencing
<b>WP</b>	Work package
<b>WWTP</b>	Wastewater treatment plant

# An Gníomhaireacht Um Chaomhnú Comhshaoil

Tá an GCC freagrach as an gcomhshaoil a chosaint agus a fheabhsú, mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaoil a chosaint ar thionchar díobhálach na radaíochta agus an truaillithe.

## Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

**Rialáil:** Rialáil agus córais chomhlíonta comhshaoil éifeachtacha a chur i bhfeidhm, chun dea-thorthaí comhshaoil a bhaint amach agus díriú orthu siúd nach mbíonn ag cloí leo.

**Eolas:** Sonraí, eolas agus measúnú ardchaighdeán, spriocdhírthe agus tráthúil a chur ar fáil i leith an chomhshaoil chun bonn eolais a chur faoin gcinnteoireacht.

**Abhcóideacht:** Ag obair le daoine eile ar son timpeallachta glaine, táirgiúla agus dea-chosanta agus ar son cleachtas inbhuanaithe i dtaobh an chomhshaoil.

## I measc ár gcuid freagrachtaí tá:

### Ceadúnú

- > Gníomhaíochtaí tionscail, dramhaíola agus stórála peitрил ar scála mór;
- > Sceitheadh fuíolluisce uirbhig;
- > Úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe;
- > Foinsí radaíochta ianúcháin;
- > Astaíochtaí gás ceaptha teasa ó thionscal agus ón eitlíocht trí Scéim an AE um Thrádáil Astaíochtaí.

### Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil

- > Iniúchadh agus cigireacht ar shaoráidí a bhfuil ceadúnas acu ón GCC;
- > Cur i bhfeidhm an dea-chleachtais a stiúradh i ngníomhaíochtaí agus i saoráidí rialáilte;
- > Maoirseacht a dhéanamh ar fhreagrachtaí an údaráis áitiúil as cosaint an chomhshaoil;
- > Caighdeán an uisce óil phoiblí a rialáil agus údaruithe um sceitheadh fuíolluisce uirbhig a fhorfheidhmiú
- > Caighdeán an uisce óil phoiblí agus phríobháidigh a mheasúnú agus tuairisciú air;
- > Comhordú a dhéanamh ar líonra d'eagraíochtaí seirbhíse poiblí chun tacú le gníomhú i gcoinne coireachta comhshaoil;
- > An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaoil.

### Bainistíocht Dramhaíola agus Ceimiceáin sa Chomhshaoil

- > Rialacháin dramhaíola a chur i bhfeidhm agus a fhorfheidhmiú lena n-áirítear saincheisteanna forfheidhmithe náisiúnta;
- > Staitisticí dramhaíola náisiúnta a ullmhú agus a fhoilsiú chomh maith leis an bPlean Náisiúnta um Bainistíocht Dramhaíola Guaisí;
- > An Clár Náisiúnta um Chosc Dramhaíola a fhorbairt agus a chur i bhfeidhm;
- > Reachtaíocht ar rialú ceimiceán sa timpeallacht a chur i bhfeidhm agus tuairisciú ar an reachtaíocht sin.

### Bainistíocht Uisce

- > Plé le struchtúir náisiúnta agus réigiúnacha rialachais agus oibriúcháin chun an Chreat-treoir Uisce a chur i bhfeidhm;
- > Monatóireacht, measúnú agus tuairisciú a dhéanamh ar chaighdeán aibhneacha, lochanna, uiscí idirchreasa agus cósta, uiscí snámha agus screamhuisce chomh maith le tomhas ar leibhéal uisce agus sreabhadh abhann.

### Eolaíocht Aeráide & Athrú Aeráide

- > Fardail agus réamh-mheastacháin a fhoilsiú um astaíochtaí gás ceaptha teasa na hÉireann;
- > Rúnaíocht a chur ar fáil don Chomhairle Chomhairleach ar Athrú Aeráide agus tacaíocht a thabhairt don Idirphlé Náisiúnta ar Gníomhú ar son na hAeráide;

- > Tacú le gníomhaíochtaí forbartha Náisiúnta, AE agus NA um Eolaíocht agus Beartas Aeráide.

### Monatóireacht & Measúnú ar an gComhshaoil

- > Córais náisiúnta um monatóireacht an chomhshaoil a cheapadh agus a chur i bhfeidhm: teicneolaíocht, bainistíocht sonraí, anailís agus réamhaisnéisiú;
- > Tuairiscí ar Staid Thimpeallacht na hÉireann agus ar Tháscairí a chur ar fáil;
- > Monatóireacht a dhéanamh ar chaighdeán an aeir agus Treoir an AE i leith Aeir Ghlain don Eoraip a chur i bhfeidhm chomh maith leis an gCoinbhinsiún ar Aerthruailliú Fadraoin Trasteorann, agus an Treoir i leith na Teorann Náisiúnta Astaíochtaí;
- > Maoirseacht a dhéanamh ar chur i bhfeidhm na Treorach i leith Torainn Timpeallachta;
- > Measúnú a dhéanamh ar thionchar pleananna agus clár beartaithe ar chomhshaoil na hÉireann.

### Taighde agus Forbairt Comhshaoil

- > Comhordú a dhéanamh ar ghníomhaíochtaí taighde comhshaoil agus iad a mhaoiniú chun brú a aithint, bonn eolais a chur faoin mbeartas agus réitigh a chur ar fáil;
- > Comhoibriú le gníomhaíocht náisiúnta agus AE um thaighde comhshaoil.

### Cosaint Raideolaíoch

- > Monatóireacht a dhéanamh ar leibhéal radaíochta agus nochtadh an phobail do radaíocht ianúcháin agus do réimsí leictreamaighnéadacha a mheas;
- > Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as tasmí núicléacha;
- > Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta;
- > Sainseirbhísí um chosaint ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

### Treoir, Ardú Feasachta agus Faisnéis Inrochtana

- > Tuairisciú, comhairle agus treoir neamhspleách, fianaise-bhunaithe a chur ar fáil don Rialtas, don tionscal agus don phobal ar ábhair maidir le cosaint comhshaoil agus raideolaíoch;
- > An nasc idir sláinte agus folláine, an geilleagar agus timpeallacht ghlan a chur chun cinn;
- > Feasacht comhshaoil a chur chun cinn lena n-áirítear tacú le hiompraíocht um éifeachtúlacht acmhainní agus aistriú aeráide;
- > Tástáil radóin a chur chun cinn i dtithe agus in ionaid oibre agus feabhsúchán a mholadh áit is gá.

### Comhpháirtíocht agus Líonrú

- > Oibriú le gníomhaireachtaí idirnáisiúnta agus náisiúnta, údaráis réigiúnacha agus áitiúla, eagraíochtaí neamhrialtais, comhlachtaí ionadaíochta agus ranna rialtais chun cosaint comhshaoil agus raideolaíoch a chur ar fáil, chomh maith le taighde, comhordú agus cinnteoireacht bunaithe ar an eolaíocht.

## Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil

Tá an GCC á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóir. Déantar an obair ar fud cúig cinn d'Oifigí:

1. An Oifig um Inbhuanaitheacht i leith Cúrsaí Comhshaoil
2. An Oifig Forfheidhmithe i leith Cúrsaí Comhshaoil
3. An Oifig um Fhianaise agus Measúnú
4. An Oifig um Chosaint ar Radaíocht agus Monatóireacht Comhshaoil
5. An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tugann coistí comhairleacha cabhair don Gníomhaireacht agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair inmí agus le comhairle a chur ar an mBord.

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