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Land use as a critical determinant of faecal and antimicrobial resistance gene pollution in riverine systems

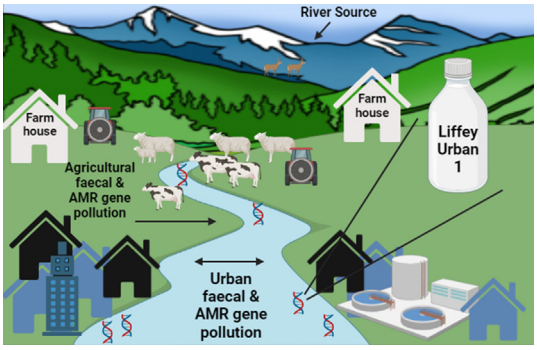
Tristan M. Nolan [a], Liam J. Reynolds [a], Laura Sala-Comorera [a], Niamh A. Martin [a], Jayne H. Stephens [a], Gregory M.P. O'Hare [b], John J. O'Sullivan [c], Wim G. Meijer [a,*]

[a] UCD School of Biomolecular and Biomedical Science, UCD Earth Institute and UCD Conway Institute, University College Dublin, Dublin 4, Ireland
[b] School of Computer Science and Statistics, Trinity College Dublin, Dublin 2, Ireland
[c] UCD School of Civil Engineering, UCD Dooge Centre for Water Resources Research and UCD Earth Institute, University College Dublin, Dublin 4, Ireland

HIGHLIGHTS

- Land use is an important determinant of faecal contamination and AMR pollution.
- AMR levels are higher in urban than in agricultural environments.
- Faecal contamination is higher in urban than in agricultural environments.
- FIB and AMR levels are correlated.
- AMR genes associated with human use were significant drivers of separation.

GRAPHICAL ABSTRACT



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ABSTRACT

The WHO recognises antimicrobial resistance (AMR) as a global health threat. The environment can act as a reservoir, facilitating the exchange and the physical movement of resistance. Aquatic environments are at particular risk of pollution, with large rivers subject to pollution from nearby human, industrial or agricultural activities. The land uses associated with these activities can influence the type of pollution. One type of pollution and a likely contributor to AMR pollution that lowers water quality is faecal pollution. Both pose an acute health risk and could have implications for resistance circulating in communities. The effects of land use are typically studied using physiochemical parameters or in isolation of one another. However, this study aimed to investigate the impact of different land uses on riverine systems. We explored whether differences in sources of faecal contamination are reflected in AMR gene concentrations across agricultural and urban areas. Water quality from three rivers impacted by different land uses was assessed over one year by quantifying faecal indicator bacteria (FIB), microbial source tracking markers (MST) and AMR genes. In addition, a multiparametric analysis of AMR gene pollution was carried out to understand whether agricultural and urban areas are similarly impacted. Faecal indicators varied greatly, with the highest levels of FIB and the human MST marker observed in urban regions. In addition, these faecal markers correlated with AMR genes. Similarly, significant correlations between the ruminant MST marker and AMR gene levels in agriculture areas were observed. Overall, applying multiparametric analyses to include AMR gene levels, separation and clustering of sites were seen based on land use characterisation. This study suggests that differences in prescription of antimicrobials used in animal and human healthcare may influence environmental resistomes across agricultural and urban areas. In addition, public health risks due to exposure to faecal contamination and AMR genes are highlighted.

* Corresponding author.
E-mail address: wim.meijer@ucd.ie (W.G. Meijer).

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1. Introduction

Antimicrobial resistance (AMR) seriously threatens global public health (Ferri et al., 2017; Marston et al., 2016; Shallcross et al., 2015). It is predicted that AMR could cause up to 10 million deaths per year by 2050 (O'Neil, 2016). Although the accuracy of this prediction has been debated, in 2019, AMR was responsible for more deaths than HIV/AIDS and malaria, and an estimated 4.95 million deaths were associated with drug-resistant infections. Of those, approximately 25 % of the mortality was directly linked to drug resistance (Collaborators, 2017; Murray et al., 2022). The rise in AMR has been attributed to several factors, including poor antimicrobial stewardship in human medicine, prophylactic use of antimicrobials in agriculture and lack of public awareness (Dadgostar, 2019; File Jr. et al., 2014; Musoke et al., 2021; Prestinaci et al., 2015). These all contribute to increasing levels of resistance circulating in the wider environment.

A "One Health" approach recognises the connection between human, animal and environmental health and is likely to be a central tenet to any strategy that successfully contributes to reductions in AMR in the environment (Mackenzie and Jeggo, 2019; Thakur and Gray, 2019). Components of the One Health strategy concentrate on water, sanitation, and hygiene (WASH), all central to AMR transmission. Further to these components, a significant focus on waste management is needed to reduce the spread of AMR, as inadequate disposal of antimicrobial waste contributes to higher levels of AMR in the environment (Prestinaci et al., 2015). Antimicrobial waste can be detrimental to local ecosystems and is now being viewed as an emerging environmental pollutant (Kraemer et al., 2019). Therefore, to properly deal with this exigent problem, there exists a need for both increased surveillance and transparent information on the safe levels of antimicrobial resistant microbes, antimicrobials and antimicrobial resistance genes in the environment (Programme, 2022).

Faecal contamination of waterbodies is a major cause of deteriorating water quality and an important route by which AMR enters the environment (Faldynova et al., 2013; Ho et al., 2020; Kim and Cha, 2021; Reynolds et al., 2020; Sala-Comorera et al., 2021a). Depending on the class of antimicrobials, up to 90 % of an administered dose can be excreted in faeces (Polianciuc et al., 2020). Consequently, faecal pollution increases the likelihood of mixing with environmental pollutants and poses a severe health risk if consumed via food and water webs. Recurrent exposure to these pollutants has been associated with multiple chronic conditions (Ianiro et al., 2016). Worryingly, some studies have also found that recreational users of water are regularly being exposed to antibiotic resistant micro-organisms, and this underlines the need to understand how and when pollution is arising (Leonard et al., 2015; O'Flaherty et al., 2019).

There are many sources of aquatic environmental pollution, and these are typically driven by human, industrial or agricultural activities. The land uses associated with these activities can also influence the type of pollution observed in nearby waterbodies. In urban areas, discharging wastewater plants, combined sewer overflows and from diffuse sources in agricultural settings are potential sources (Ma et al., 2016; Murray et al., 2022). Therefore, the spatial location and source of the pollution input can impact differently the waterbodies into which they discharge. Water bodies, such as rivers, may span large geographic areas, with several defined but different land use classes within the catchment. Headwater streams (1st and 2nd order) typically rise in upland areas, often in areas characterised by peatland and bog. Downstream, the pressures change as rivers flow through agricultural and urban areas, and water quality is now impacted directly by agricultural and anthropogenic activities (Conroy et al., 2016; Mangialajo et al., 2007). These differences in the biological sources of faecal pollution change the inherent health risk to the public (Dwight et al., 2004; Giri and Qiu, 2016). In addition to the changes in risk, a shift in pollution source may lead to changes in AMR gene pollution because of discrepancies in the prescription and consumption of antimicrobials most frequently used in Ireland in medicine and veterinary medicine.

As the effects of land use activities on overall water quality are typically studied using physiochemical parameters or in isolation from one another – more understanding is needed regarding the dynamics of faecal and AMR gene pollution across bodies of water impacted by both urban and agricultural activities. Here we investigate the impact of land use on nearby water body quality and explore whether differences in sources of faecal contamination are reflected in AMR gene concentrations. The AMR genes chosen for this study confer resistance to the ciprofloxacin, sulphonamides, tetracyclines and beta-lactam classes of antimicrobials, which are important across Ireland in medicine and veterinary medicine. In addition, through a multiparametric analysis of AMR gene pollution, we explore whether riverine systems subject to agricultural and urban pollution sources behave similarly and indicate specific land use pressures.

2. Materials and methods

2.1. Study area and sampling design

Dublin Bay is a designated UNESCO Biosphere and home to several designated bathing waters. The River Liffey, Tolka and Dodder are three of the largest rivers in Dublin. The Greater Dublin population is c. 2.1 million people and equates to just over 40 % of the Republic of Ireland's population.

The River Liffey rises in the Wicklow mountains and stretches 125 km, passing through peat and bog land, through agricultural lands in County Kildare and into the urban conurbation of Dublin before discharging into Dublin Bay. The catchment area of the River Liffey is c. 1250 km^2. The River Dodder, with a catchment area of c. 120 km^2, rises near the River Liffey in the Wicklow mountains, but with a stream length of 26 km, takes a more direct route through agricultural lands before entering Dublin Bay from the south of the city. The River Tolka, with a catchment area of c. 148 km^2, originates in agricultural land areas of County Meath and flows for a length of c. 22 km through agricultural lands and the urban fabric of Dublin City before discharging to the northern side of Dublin Bay (Fig. 1).

Over a one-year period from May 2019 to May 2020, fortnightly water samples were collected (n = 212) from fourteen sites across the catchments of the Rivers Liffey, Dodder and Tolka (Fig. 1; Supplementary Table 1). Seven sampling locations were included on the River Liffey, one in peatland, four in agricultural and two in urban areas; four locations were sampled in the River Tolka, two in agricultural and two in urban settings; and three locations were sampled in the River Dodder, one each in peatland, agricultural and urban settings. The designation of land use was based on the Irish EPA Co-ORdinated INformation on the Environment (CORINE) land use maps. Grab samples were collected in duplicate in autoclaved sterile one litre bottles, refrigerated at 4 °C and processed within 24 h.

2.2. Enumeration of faecal indicator bacteria

Faecal indictor bacteria (FIB), *E. coli* and intestinal enterococci were enumerated by water filtration of up to 100 ml of sample. *E. coli* (ISO 16649-1:2001) and intestinal enterococci (ISO 7899-2:2000) levels/concentrations were determined for all samples. Sample was processed and filters were incubated on Tryptone Bile X-Glucuronide agar (Sigma-Aldrich, USA) for *E. coli* and on Slantez and Bartley (SB) & bile aesculin agar (BAA) for intestinal enterococci. The mean CFU/100 ml was calculated for each site on each sampling date.

2.3. Extraction of DNA

Water samples of 100 ml volume were concentrated via 0.22 μm nitrocellulose filter membrane filtration. Filters were transferred into single use polypropylene tubes containing ice cold lysis buffer (5 M guanidine isothiocyanate, 100 mM EDTA [pH 8.0], 0.5 % [*w/v*] sodium lauroyl sarcosinate) and stored at −20 °C until extraction. Extraction was carried out using a previously modified protocol using a Qiagen DNeasy Blood and Tissue kit (Qiagen, Germany) (Gourmelon et al., 2007).

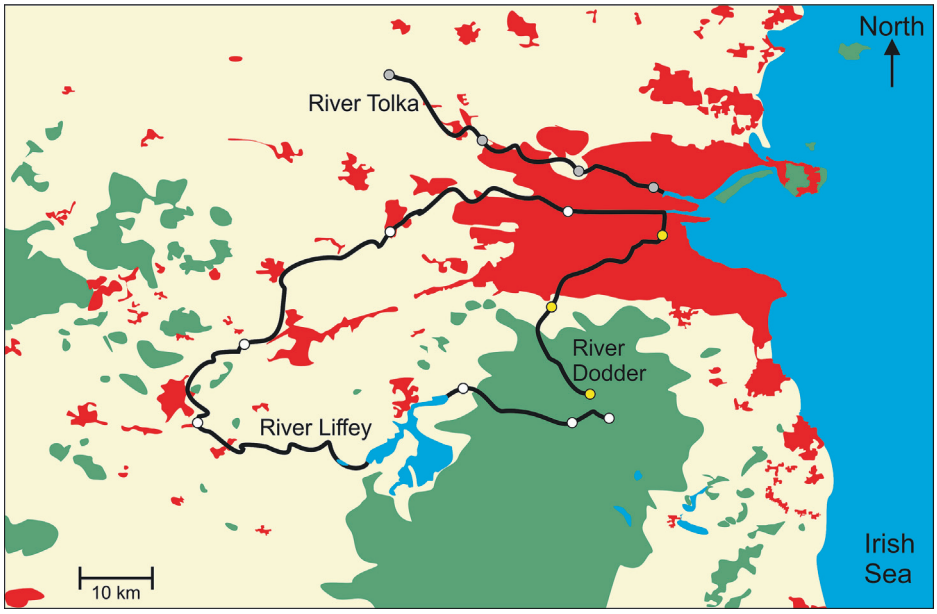


Fig. 1. Sampling sites on rivers. Fourteen sampling points across three rivers in Leinster, Ireland. River Liffey (White), River Tolka (Grey) and the River Dodder (Yellow). Colours on the base map indicate land use classification via the EPA Co-ORdinated INformation on the Environment. Green indicates peatland/bog/wetlands and nature reserves, yellow indicates agricultural, and red indicates urban areas.

2.4. MST and AMR gene quantification

The bla_{TEM}, *qnr*S, *tet*(O) and *sul*1 AMR genes and the human (HF183) and ruminant (CF128) microbial source tracking (MST) markers were quantified on the Roche Lightcycler 96 platform (Bernhard and Field, 2000). SYBR Green 1 (Roche Diagnostics, Switzerland) fluorochrome was used and all qPCR cycle conditions included a 10 min pre incubation at 95 °C for 10 min and a subsequent melt curve analysis (Supplementary Table 2). Targeted primers and qPCR amplification conditions were used to amplify DNA targets and both AMR and MST genes were expressed as gene copies per 100 ml (gc/100 ml).

MST genes and AMR PCR targets were cloned into a pBLUE and pGEM-T easy plasmid, respectively. Plasmid DNA was subsequently extracted using Qiagen's QIAprep spin miniprep kit. Circular plasmid DNA was linearised and DNA concentrations were determined using Qubit. Using the linearised plasmids, standard curves between 10 and 10^6 gc were generated for each assay. Each assay efficiency was determined using the eq. $E = 10^{\left(\frac{1}{slope}\right)} - 1$ (Rutledge and Côté, 2003). All standard curves had an R^2 value >0.985. The limit of quantification (LOQ) was determined as the lowest concentration of target DNA quantified, with no more than a 0.5 standard deviation of the $\log_{10}$ concentration as previously described. The limit of detection (LOD) of the qPCR was determined as the lowest number of nucleic acid targets in each sample volume, detected in at least 95 % of the replicates (Supplementary Table 1) (Rutledge and Stewart, 2008).

2.5. Data analysis

Univariate analysis was carried out using GraphPad Prism 10 software. A significance cut-off of $p \leq 0.05$ was used for all analyses. Multivariate analyses were carried out on RStudio 3.5.1 and SPSS 27.0.1. Differences in land use were explored using a multivariate analysis of variance (MANOVA) using the EPA CORINE land use characterisation as fixed factors and genes encoding the β-lactam, sulfonamides, tetracycline, and fluoroquinolone genes as response variables. Subsequent univariate analysis of variance (ANOVA) with Dunn's post hoc analysis was used on significant differences found between land uses. Spearman correlation was used to identify relationships between faecal indicators and AMR genes. All data was $\log_{10}$ transformed and a significance cut off $p < 0.05$ was used.

Descriptive multivariate analyses were carried out to highlight differences among land impacted rivers. All data were normalised via log transformation to reduce the impact of outliers. Hierarchical clustering was achieved using Wards (Ward.D2) linkage with Manhattan distances (Strauss and von Maltitz, 2017). A K-means clustering statistical elbow method was used to define cluster size. A Principal Component Analysis (PCA) was performed to detect land use clusters along the reaches of the three study rivers. A scree plot was used to indicate the appropriate number of principle components to adequately summarise the data which explains the bulk of the variation.

3. Results

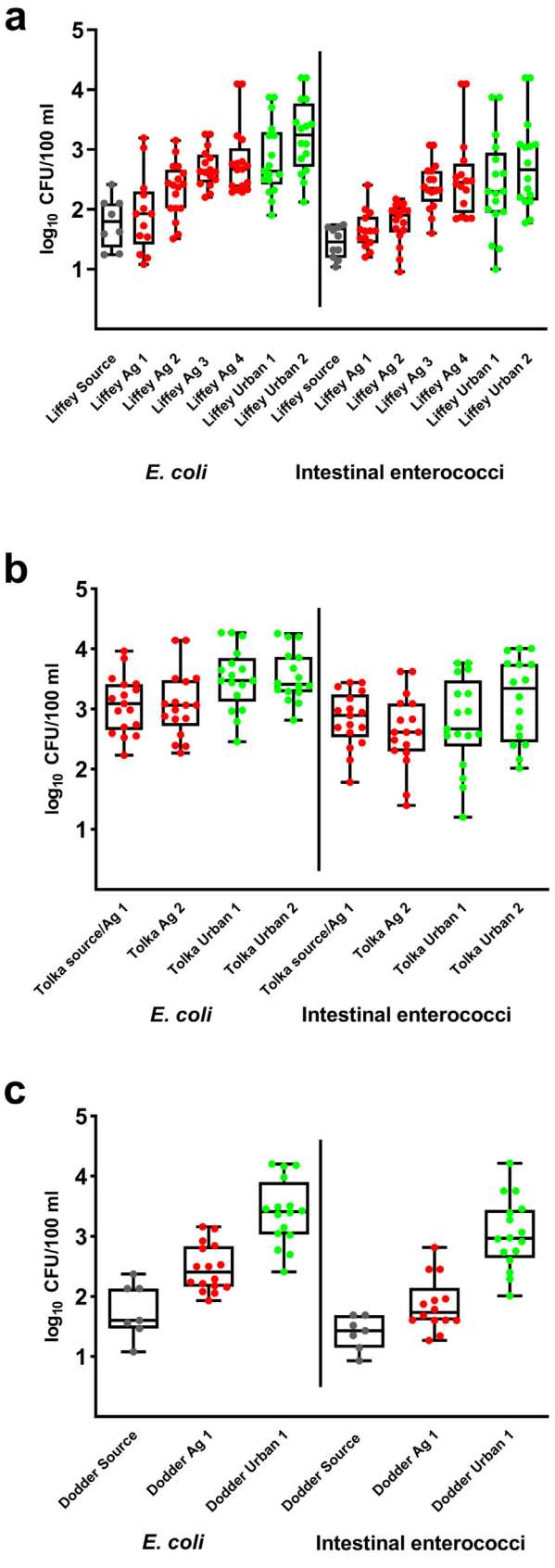
3.1. High and increasing levels of faecal pollution across three rivers - peaking in urban impacted areas

The lowest levels of *E. coli* and intestinal enterococci were observed at the source sampling points for the Rivers Dodder and Liffey, located in Kippure, Co Wicklow (Fig. 1). Here, *E. coli* levels varied over two orders of magnitude, while intestinal enterococci ranged over one order of magnitude and FIB were positive in 55 % of the samples (n = 30). An increase in the levels of FIB and sample positivity were observed in downstream agricultural sampling points for the Rivers Dodder and Liffey, where 93.5 % of samples were positive for FIB, with levels more than an order of magnitude higher than at the source (where n = 84).

In contrast to the Rivers Dodder and Liffey, which originate in the Wicklow mountains, the source of the River Tolka is in the agricultural area of Dunshaughlin, County Meath. All samples (n = 16) were positive for the presence of FIB. While FIB levels ranged over three orders of magnitude, the median value was two orders of magnitude higher than that observed at the sources of the Rivers Dodder and Liffey (Fig. 2).

An increase in the levels of faecal pollution was observed in all three rivers as they became increasingly impacted by urban influences. The median concentrations of FIB increased by over two orders of magnitude between the sampling points at the source and those in the urban locations in the

Rivers Liffey and Dodder, with a single order of magnitude difference being observed between agricultural and urban settings. The number of samples that were positive for the presence of FIB increased at urban sampling points in the Rivers Dodder and Liffey, whereas all samples from the River Tolka were positive for the presence of FIB. In urban sampling points across all three rivers, FIB ranged over four orders of magnitude and 100 % of the samples were positive for FIB (n = 82). The highest median concentrations for both *E. coli* and intestinal enterococci were sampled in urban locations in Dublin City in the three studied rivers (Fig. 2).



Variations in the human MST marker across samples varied over two orders of magnitude, whereas the ruminant MST was shown to vary over a one order of magnitude at the source sampling points in the Rivers Dodder (n = 15) and Liffey (n = 15), which are separated by approximately 100 m. At this location, 26.7 % of the total number of samples (Rivers Liffey and Dodder, n = 30) were positive for the human MST marker and 13.3 % were positive for the ruminant MST marker (Fig. 3).

Water samples taken in agricultural areas, obtained from the rivers Liffey and Dodder, showed a strong increase in the positivity rate for human and ruminant MST markers, with 61.5 % of samples positive for the human and 91.5 % of samples positive for the ruminant marker (Rivers Liffey and Dodder, total n = 84). The human MST marker ranged over three orders of magnitude, while the ruminant MST marker ranged over four orders of magnitude. In contrast, samples taken from the source of the River Tolka had higher levels and positivity rates for both MST markers than at the source of the Rivers Dodder and Liffey. The human MST marker ranged over two orders of magnitude, while the ruminant MST marker ranged over three orders of magnitude, with 81.3 % of the samples being above the limit of detection for both the human and ruminant MST markers (n = 16).

Across the three rivers, both MST markers varied by an order of magnitude between the sampling points in agricultural and urban settings. In urban areas, the ruminant MST marker ranged by over six orders of magnitude, with 53.1 % of the samples being above the detection limit (n = 84). In agricultural settings, the ruminant MST marker varied over seven orders of magnitude and was found in ~90 % of the samples. The human MST marker in the three rivers ranged by over five orders of magnitude in urban areas, similar to what was observed in agricultural areas. The highest absolute human MST marker levels and positivity rates in the studied rivers were observed in samples taken from urban settings, with 98.2 % (compared to c. 70 % for agricultural settings) of the samples being above the limit of detection (n = 82) (Fig. 3).

3.2. Increasing levels and prevalence of antimicrobial resistance genes observed in riverine systems - highest prevalence in urban areas

The lowest levels of positivity of all four resistance genes were observed at the source sampling points in the Rivers Dodder and Liffey (Fig. 4). Both *qnr*S and *sul*1 ranged over five orders of magnitude, while *tet*(O) and bla_{TEM} ranged over four orders of magnitude, with positivity rates of 46 %, 43.3 %, 56.7 % and 33.3 % for *qnr*S, *sul*1, *tet*(O) and bla_{TEM} at the source sampling points, respectively. All four AMR genes were in the same order of magnitude at the downstream agricultural sampling points on the Rivers Dodder and Liffey. An increase in the level of positivity were found in samples taken from agricultural areas compared to source sampling points, with 70.2 %, 63.1 %, 86.4 % and 85.7 % of the samples being above the detection limit for *qnr*S, *sul*1, *tet*(O) and bla_{TEM}, respectively (n = 84).

In comparison to the Rivers Liffey and Dodder, the source of the River Tolka exhibited higher positivity for *qnr*S, *sul*1, *tet*(O) and bla_{TEM} resistance genes -with 87.6 %, 87.6 %, 100 % and 93.8 % of the samples being above the detection limit for *qnr*S, *sul*1, *tet*(O) and bla_{TEM}, respectively. In addition, *tet*(O) and bla_{TEM} were two orders of magnitude higher, while *qnr*S and *sul*1 were within a single order of magnitude (n = 16).

Fig. 2. Faecal pollution in three freshwater rivers. Shown are the faecal indicators for (a) the River Liffey, (b) the River Tolka and (c) the River Dodder. Each river has multiple locations categorised under a specific land use, as per EPA Corine land use. Each graph shows *E. coli* for all sampled points on each river, followed by intestinal enterococci. Sampling occurred over a one-year period (n = 212). The box plot depicts the median value, the 10th and 90th percentile and faecal indicator bacteria. Only samples that tested positive are shown on the boxplot.

In all three rivers, an increase in levels of positivity for all four AMR genes was found in urban areas. The AMR genes, *qnr*S, *tet*(O), bla_{TEM} and *sul*1 all ranged over five orders of magnitude (n = 82) (Fig. 4) with 89.2 %, 91.6 %, 92.8 % and 89.2 % of samples testing positive for *qnr*S, *tet* (O), bla_{TEM} and *sul*1, respectively.

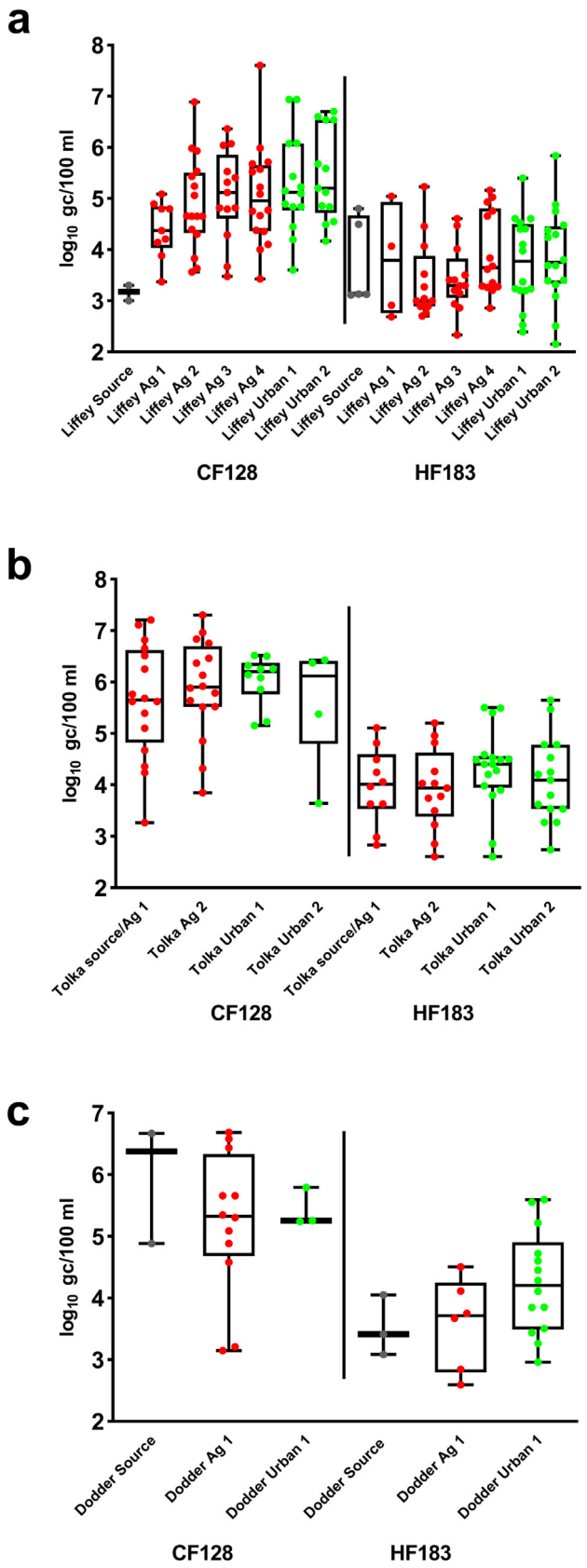


Fig. 3. Biological sources of faecal pollution in freshwater rivers. Microbial source tracking markers in freshwater rivers. Shown are the human associated faecal marker (HF183) and ruminant associated marker (CF128) for (a) the River Liffey, (b) the River Tolka and (c) the River Dodder. Each river has multiple locations categorised under a specific land use, as per EPA Corine land us. Each graph shows first the ruminant marker, followed by the human associated marker. Each graph shows the levels of faecal markers over one year (n = 212). The box plot depicts the median value, the 10th and 90th percentile and faecal indicator values outside of this range shown as black circles. Only samples that tested positive are shown on the boxplot.

3.3. Faecal contamination correlates with antimicrobial resistance genes in riverine systems

Across all 14 sampling points on the three studied rivers, we observed high levels of significant correlation between all four faecal markers and AMR genes (Table 1). The only markers that failed to show significant correlations were the ruminant MST marker and *qnr*S. Considering the 14 sampled sites in the three rivers in the context of their dominant land uses, we begin to see varying relationships between faecal markers and AMR genes. The agricultural sites showed greater levels of correlation between the faecal markers and the AMR genes. Both *sul*1 and *qnr*S correlated significantly with all four faecal markers, whereas *qnr*S correlated with only intestinal enterococci and the human MST marker. The AMR gene bla_{TEM} was only correlated with the human MST marker. In urban locations, *tet*(O) correlated with all four faecal markers, while only *sul*1 and *qnr*S correlated with the human marker. No correlations were observed between AMR genes and faecal markers at source of the Rivers Liffey and Dodder.

3.4. The impact of land use activities – separation and clustering of land uses based on changing concentrations of antimicrobial resistance genes

MANOVA test results indicate that land use has a significant effect on concentrations of AMR genes across the three rivers (Pillai's Trace on land use factor; F-ratio = 2.31 and p-value of 0.031) (Supplementary Table 2). Subsequent ANOVA test results indicate both *qnr*S (F-ratio = 3.722, p-value of 0.026) and *sul*1 (F-ratio of 6.160, p-value of 0.002) showed significant differences among land uses. A Dunn's post hoc multiple comparison test revealed *qnr*S as significantly different between urban with both source and agriculture settings, p-value of 0.038 and a p-value of 0.016, respectively. *sul*1 showed significance between land uses, with urban settings significantly different between source and agriculture settings, p-value of 0.007. A statistically significant difference was not observed between levels of AMR genes in samples from source and agricultural settings.

Results from the hierarchical clustering, PCA and a K-means clustering further confirm the impact of land use on observed levels of AMR genes. Hierarchical clustering (Fig. 5) indicates the presence of defined clusters based on samples from urban and agricultural settings in the three rivers. Although an overlap between these clusters is shown in Fig. 5, the analysis highlights a clear grouping structure, with a principal component analysis (PCA) showing that separation is based on AMR genes (Fig. 6). The clades observed in the PCA analysis show from left to right, urban, agricultural and source locations. Two principal components were extracted accounting for c. 76 % of the total variance. PC1 explains c. 55 % of the variation that was observed. The weightings on this component were in decreasing order *sul*1, *qnr*S, *tet*(O) and bla_{TEM} (Table 2). PC2 explained 21 % of the variation seen. The weighting on these components were in decreasing order, bla_{TEM}, *tet*(O), *sul*1 and *qnr*S (Table 2). To further highlight the grouping structure of the dataset, a K-means clustering analysis grouped the full dataset into a three-group structure. These groups can be interpreted as the source, agricultural, and urban impacted areas from the colours (Fig. 7).

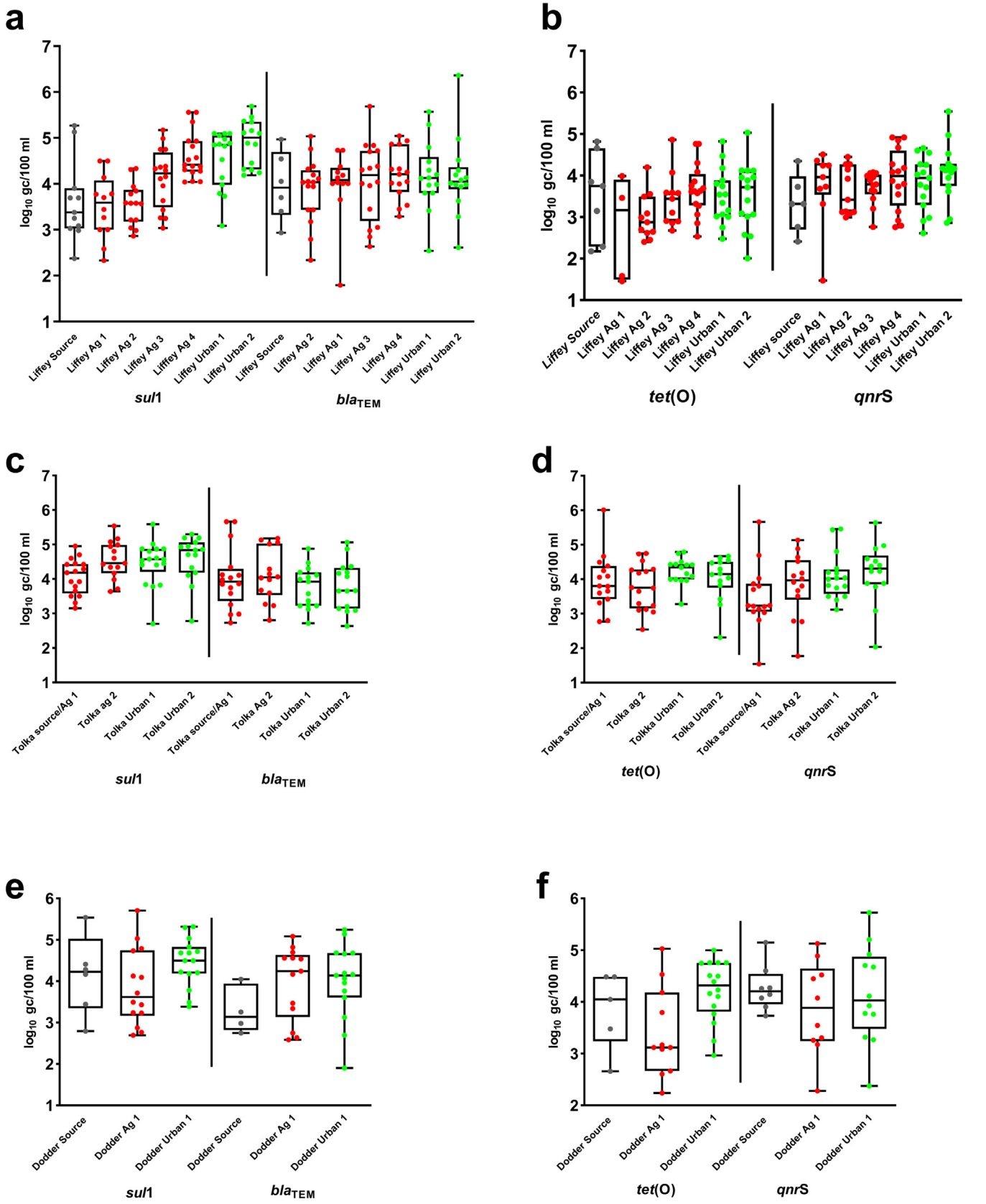


Fig. 4. Antimicrobial resistance genes in freshwater rivers. Shown are the antimicrobial resistance genes *tet*(O), *qnr*S, *sul*1 and bla_{TEM} (a), (b) in the Rivers Liffey, (c) (d) Tolka and (e) (f) the Dodder. Each river has multiple locations categorised under a specific land use, as per EPA Corine land use. Land uses peatland/bog is shown as black, agriculture as red and urban as green. Each graph shows the levels of faecal markers over one year (n = 212). The box plot depicts the median value, the 10th and 90th percentile. Only samples that tested positive are shown on the boxplot.

Table 1
Correlation between faecal indicator markers and AMR genes in all three rivers.

River	Land use	FIB/MST marker	bla_{TEM}	*sul*1	*qnr*S	*tet*(O)
Liffey	All	*E. coli*	**0.15***	**0.41*****	**0.29*****	**0.46*****
Tolka		IE	**0.18****	**0.42*****	**0.30*****	**0.44*****
Dodder		Human	**0.23*****	**0.44*****	**0.31*****	**0.58*****
		Ruminant	**0.17****	**0.23*****	0.12	**0.36*****
	Agricultural sites	*E. coli*	0.02	**0.37*****	0.15	**0.42*****
		IE	0.11	**0.46*****	**0.20***	**0.38*****
		Human	**0.24****	**0.39*****	**0.27****	**0.55*****
		Ruminant	0.10	**0.28****	0.01	**0.50*****
	Urban sites	*E. coli*	−0.16	−0.04	0.10	**0.29****
		IE	−0.17	−0.01	0.10	**0.27***
		Human	0.02	**0.33****	**0.22***	**0.52*****
		Ruminant	−0.01	0.10	0.14	**0.26***
	Sources	*E. coli*	0.05	0.08	0.04	−0.21
		IE	0.26	0.19	0.09	−0.12
		Human	0.15	0.20	−0.04	0.14
		Ruminant	0.13	0.29	0.08	0.19

* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$.

4. Discussion

The main aims of this study were to understand the effects of faecal pollution on the lowering of water quality and its relationship with AMR gene pollution across lotic systems. Moreover, we aimed to investigate the impact of land use and analyse if changes in antimicrobial resistance occur with respect to agricultural and anthropogenic activities. Our study shows an increase in the prevalence and abundance of AMR genes as these systems

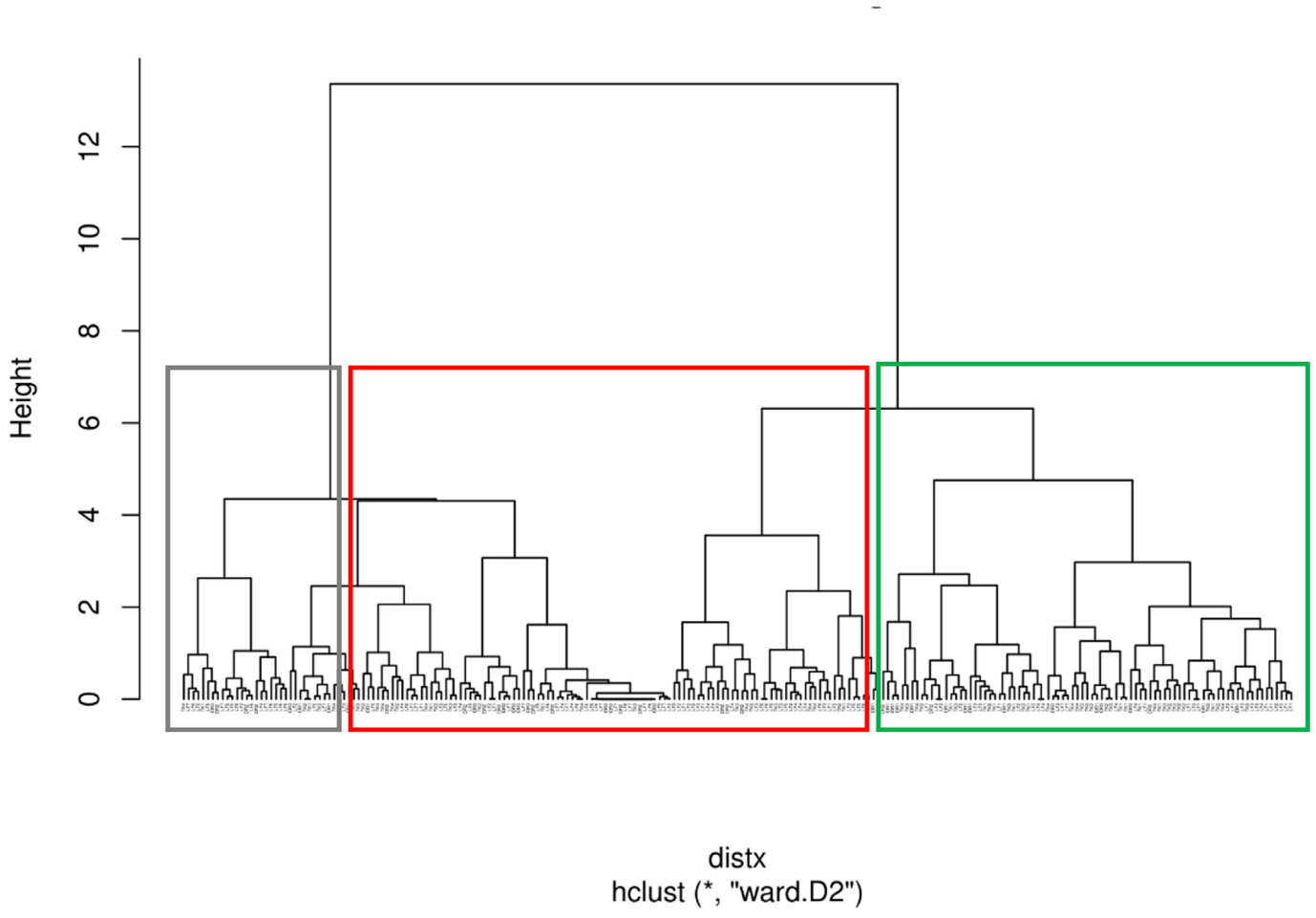


Fig. 5. AMR gene hierarchical clustering of sampling sites. Hierarchical clustering of river sites using changing concentrations of antimicrobial resistance genes, *tet*(O), *qnr*S, *sul*1 and bla_{TEM} used. Fourteen locations on the Rivers Liffey, Dodder and Tolka, were sampled over a year (n = 212). Wards (Ward.D2) linkage with Manhattan distances applied. Three coloured boxes indicate where most of the samples are clustering. Land use of peatland/bog/forestry and nature reserves from the Rivers Liffey and Dodder shows black agriculture as red and urban as green (n = 212).

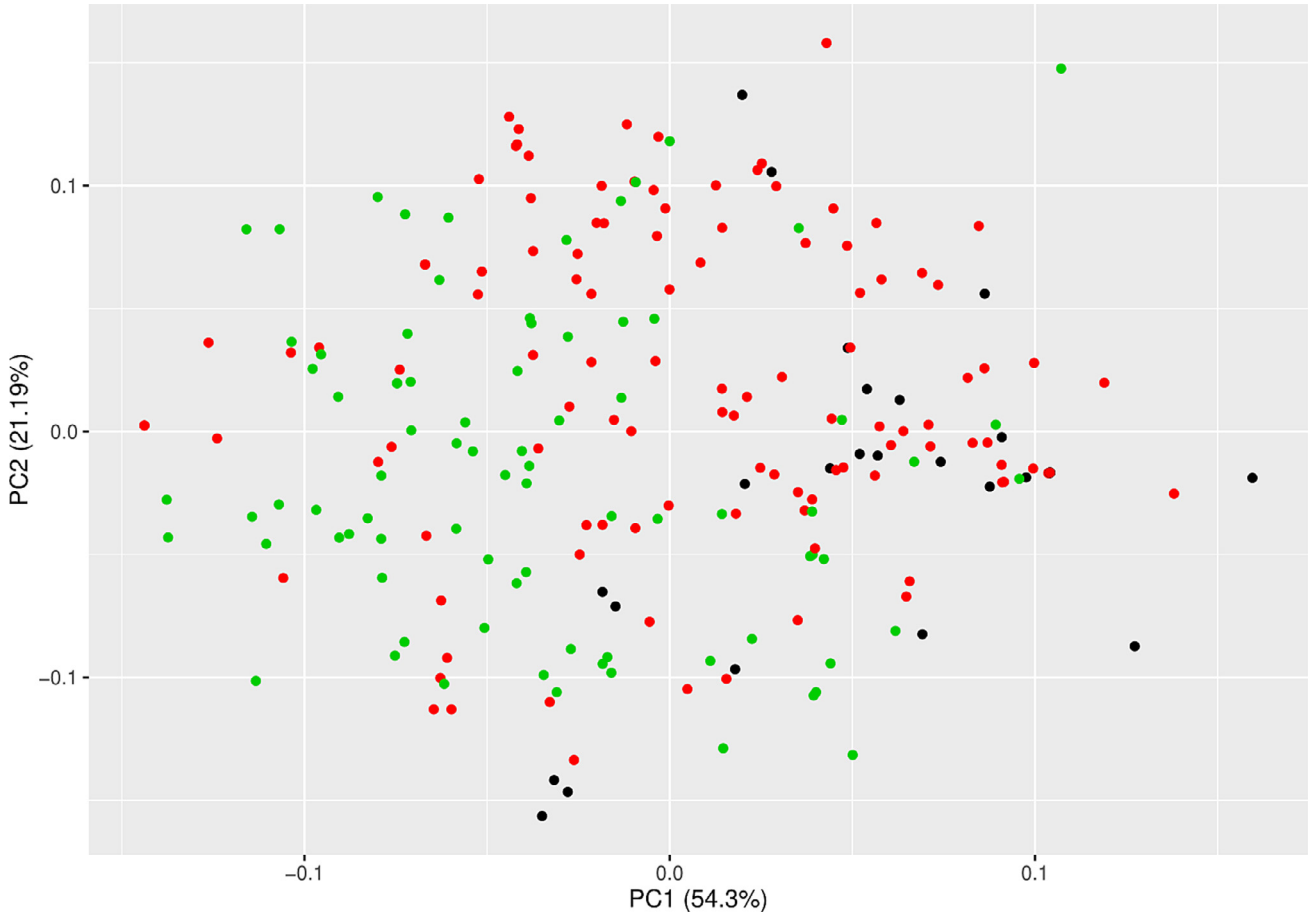


Fig. 6. Principle component analysis using AMR gene concentrations in freshwater rivers. Principal component analysis using changing concentrations of AMR genes for the Rivers Liffey, Dodder and Tolka. Each river has multiple locations categorised under a specific land use, as per EPA Corine land use. Land use of peatland/bog/forestry and nature reserves from the Rivers Liffey and Dodder is shown as black, agriculture as red and urban as green (n = 212).

move from their respective sources through agriculture and urban areas. Furthermore, the data obtained during this study showed a steady increase in faecal pollution, increasing from the source sampling points and peaking in urban locations. Additionally, the increase in faecal pollution was compounded by significant correlations with AMR genes across the three rivers. Results suggest that locations grouped based on their environmental bio-geographical properties behaved more akin to similarly impacted areas. Significant differences between land uses were observed when looking at changes in concentrations of AMR genes. These results align with other work that viewed the effect of land use on water quality using physiochemical, organic matter and nutrient data as parameters on riverine ecosystems (Le et al., 2021; Mello et al., 2020; Stets et al., 2020; Verhougstraete et al., 2015; Zhou et al., 2020).

It is important to note that AMR is a natural microbial tool in ecological adaptation and a result of unavoidable evolutionary processes. AMR genes have been found in remote environments, such as polar regions and alpine environments, with minimal human activities (Rogers et al., 2018; Scott et al., 2020). AMR genes were found at the source sampling sites, a location with minimal influence from agriculture and urban activities. However, both the frequency of detection and concentrations were lower than at downstream sampling locations in agricultural and urban settings. The presence of AMR genes at the sources of these rivers could be attributable to the presence of wild animals, such as deer, sheep, and birds (Ballash et al., 2021; Scott et al., 2012; Smith et al., 2014). In addition, river sources are typically located in upland areas that are popular recreational hiking spots, and this potentially exposes these sites to other contaminations. Furthermore, animal and human influences were reflected in levels of human and ruminant MST markers found sporadically at our source locations.

The strong correlative relationship between faecal indicators and AMR genes suggests faecal contamination as a contributor to AMR in the environment. The intestinal microbiota and, thus, bacteria have been shown to harbour AMR genes and determinants and may be a direct source of these environmental pollutants (Barrera et al., 2019; Casals-Pascual et al., 2018). Faecal pollution of rivers can occur in various ways, such as point sources like sewer misconnections and combined sewer overflows that can discharge untreated sewage into rivers and streams (Hill et al., 2006; Olds et al., 2018). Other water bodies may be subject to diffuse faecal pollution, such as that from run-off from agricultural settings (Fernández-Alvarez et al., 1991). In addition to faecal pollution, land use activities can also contribute to other forms of pollution of lotic systems. Heavy metals, biocides and quaternary ammonium compounds have been found in pollution point sources such as combined sewer overflows, which impact water quality and pose a health risk to the public (Paijens et al., 2021; Van de Voorde et al., 2012; Xu et al., 2018).

Table 2
Loadings matrix of the specified principal components.

	PC1	PC2
*qnr*S	−0.48218	0.044044
tet(O)	−0.44573	−0.59176
*sul*1	−0.63896	−0.11959
bla_{TEM}	−0.40069	0.795976

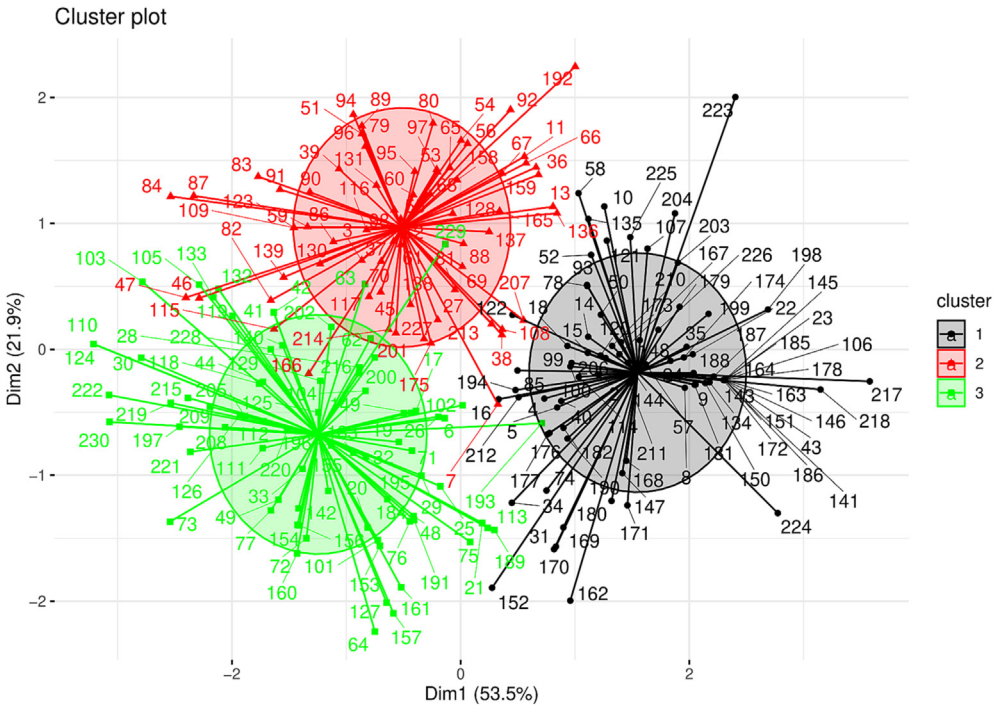


Fig. 7. K-means three group structure of sampling sites. K-means clustering is shown. A K-means clustering statistical elbow method was used to define cluster size. Each river has multiple locations categorised under a specific land use, as per EPA Corine land use. Land use of peatland/bog/forestry and nature reserves from the Rivers Liffey and Dodder is shown as black, agriculture as red and urban as green (n = 212).

Another possible contributor to the increased levels of AMR gene pollution in downstream sampling points and the significant differences observed across land uses may be a result of the variation of antimicrobials consumed in human and agricultural medicine. The AMR genes from the sulphonamides and fluoroquinolone classes of antimicrobials were significant drivers across land uses. In Ireland, sulphonamides and fluoroquinolone rank in the top five classes of antimicrobials used in human medicine. Whereas in veterinary antibiotics supplied to Ireland in 2018, sulphonamides and trimethoprim make up 20.7 % of sales- while fluoroquinolones only accounted for 0.9 % of sales (DOH, 2018). Therefore, this suggests that the prominent use of certain classes of antimicrobials in human medicine may influence differences in the resistome observed across agricultural and urban areas, with weighted differences in the consumption of key antimicrobials across human and animal medicine leading to higher abundances of certain AMR genes associated with that type of pollution. This lends credence to why others view the *sul*1 AMR gene as a possible proxy marker for anthropogenic pollution (Cacace et al., 2019; Czekalski et al., 2015; Reynolds et al., 2020). In addition, the effect of regional selection pressure on resistance patterns provides unique opportunities. Data on localised environmental resistance profiles in sewage has been shown to reflect clinical patterns, and thus, the analysis of environmental resistance could help predict regional resistance problems in clinical cases across human and animal medicine (Aarestrup and Woolhouse, 2020; Larsson and Flach, 2022; Pärnänen et al., 2019).

These results add to, and highlight, the problem of faecal pollution as a major driver of poor microbial water quality (Bain et al., 2014; Paruch et al., 2019; Sala-Comorera et al., 2021b). The levels of faecal pollution observed can lead to an inherent health risk to recreational users (Cabelli et al., 1982; Cheung et al., 1990; Donovan et al., 2008; Kay et al., 1994; Prüss, 1998). The concentration of faecal indicator bacteria detected across all three rivers was frequently above the no-observed adverse effect levels (NOAELs) for both *E. coli* (100 CFU/100 ml) and intestinal enterococci (25 CFU/100 ml) (Wiedenmann et al., 2006). The public and recreational users of rivers are therefore, at risk of direct and indirect exposure to human and animal pollution and potential exposure to antimicrobial resistant microbes, pathogens, and active antimicrobials (Leonard et al., 2022; Marshall and Levy, 2011). In addition, the global use of freshwater as a source of drinking water further highlights potential exposure to antimicrobial polluted waters. Exposure to this type has been linked to adverse changes in the gut microbiome, with more research needed to understand the short- and long-term effects (Ianiro et al., 2016; Kraemer et al., 2019; Yang et al., 2021).

5. Conclusion

The main aims of this study were to understand the effects of faecal pollution on the lowering of water quality and its relationship with AMR gene pollution across lotic systems impacted by different land uses. AMR genes were found at the source sampling sites, a location with minimal influence from agriculture and urban activities. The presence of AMR genes at the sources of these rivers could be attributable to the presence of wild animals, such as deer, sheep, and birds. The strong correlation between faecal indicators and AMR genes proposes faecal contamination as a contributor to AMR in the environment. Another possible contributor to the increased levels of AMR gene pollution in downstream sampling points and the significant differences observed across land uses may result from the variation of antimicrobials consumed in human and agricultural medicine. The AMR genes from the sulphonamides and fluoroquinolone classes of antimicrobials were significant drivers across land uses. The levels of faecal and AMR pollution observed can lead to an inherent health risk to recreational users. With recreational users at risk of exposure to human and animal pollution and potentially antimicrobial resistant microbes and pathogens.

CRediT authorship contribution statement

Tristan M. Nolan: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Liam J. Reynolds:** Investigation, Validation. **Laura Sala-Comorera:** Validation. **Niamh A. Martin:** Investigation. **Jayne H. Stephens:** Investigation. **Gregory M.P. O'Hare:** Writing – review

& editing. **John J. O'Sullivan:** Writing – review & editing. **Wim G. Meijer:** Conceptualization, Supervision, Writing – review & editing, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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