Correlation between antimicrobial resistance and faecal contamination in small urban streams and bathing waters

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HIGHLIGHTS

• All faecal indicators and ARGs correlated in the wastewater impacted Liffey Estuary
• Urban streams were intermittently impacted by high levels of faecal indicators.
• ARGs and faecal indicators sporadically correlate in small urban streams.
• Faecal indicators and ARGs sporadically correlated in urban bathing waters.
• Human, gull and dog source tracking markers were quantified in urban bathing waters.

GRAPHICAL ABSTRACT

Abstract

Antibiotic resistance represents the greatest challenge to healthcare systems around the world. As antibiotic resistance genes (ARGs) are shed in faeces, many studies have focused on how wastewater effluent contributes to ARG pollution in rivers. However, small urban streams and bathing waters not impacted by treated wastewater have received little attention though they may be important reservoirs of ARGs. The main objective of this study was to assess the extent to which ARG and faecal pollution impact small urban streams and bathing waters and to determine if there is a relationship between these contaminants. For one year, bi-monthly water samples were collected from two urban streams and Dublin city’s three designated bathing waters. The Liffey Estuary, that receives treated wastewater, was also sampled. The sul1, tet(O), qnrS, blaTEM, blaSHV and blaCTX-M ARGs were quantified. E. coli and intestinal enterococci levels were determined and the source of faecal pollution (human, dog, gull) quantified by microbial source tracking. Our results show that the Liffey Estuary, the urban streams and the bathing waters are highly impacted by ARGs and human faeces. There were clear correlations between all of the studied faecal indicators and ARGs in the Liffey Estuary. In the urban streams relationships were observed for only some of the ARGs and faecal indicators, which is likely a result of non-continuous sewage leaks and overflows to the streams. Similarly, only some ARGs correlated with faecal indicators in the urban bathing waters. The source of ARGs in the bathing waters is likely to be multifaceted as we detected sporadic dog and gull faecal markers. This study demonstrates that small urban streams and bathing waters are reservoirs of ARGs and that they may pose a previously unrecognised public health risk as they have the potential to transmit enteric pathogens and antibiotic resistance determinants.

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1. Introduction

Since their introduction, antibiotics have become a pillar of modern medicine. However, with the discovery of each new antibiotic, resistance to it has followed. It is now estimated that over 33,000 deaths are attributable to antibiotic resistant infections each year in Europe alone and that globally 10 million deaths a year may result from antibiotic resistance by 2050 (O’Neill, 2016; Cassini et al., 2019).

Antibiotics and antibiotic resistance genes (ARGs) are shed in faeces, thus, sewage can contaminate receiving water with ARGs. A recent survey of metagenomic datasets demonstrated that faecal pollution can explain ARG levels in waters impacted by faeces pollution (Karkman et al., 2019). These ARGs are a public health risk as they may be harboured by pathogenic or commensal bacteria that upon ingestion, can transfer their ARGs to gut microbiota by horizontal gene transfer (Huddleston, 2014). In fact, a metagenomic study identified overlaps in the resistomes of human pathogens and environmental bacteria, indicating that this route of ARG transfer does occur (Pehrsson et al., 2016). The increasing frequency of infections caused by antibiotic resistant pathogens is associated with increased mortality and is recognised by the World Health Organisation as a global threat (WHO, 2018). In recent times there has been an increased awareness that ARGs in water is an emerging threat, especially within the concept of One Health, which acknowledges that animal, human and environmental ‘health’ are connected (Marti and Bakázar, 2013a).

Most studies that have looked at antibiotic resistance in urban rivers have focused on large river systems receiving discharge from wastewater treatment plants (WWTPs). Additionally, many of these studies were carried out in countries with high antibiotic consumption, including China and India, although more recently European rivers have been studied (Marathe et al., 2017; Zhou et al., 2017; Próia et al., 2018). Small urban streams not receiving treated sewage are overlooked as sources of ARG pollution, although they may be a public health risk in this regard. Baral et al. determined that storm drain outfalls were the largest contributor of ARGs to the small urban Antelope creek (United States), likely resulting from leakages in wastewater pipes (Baral et al., 2018). As urban rivers and streams flow through densely populated areas they may be impacted on by faecal pollution from treated waste, sewer overflows and misconnected and leaking sewage pipes (Kay et al., 2008; Kapoor et al., 2013). Elaborating on this, a number of studies have shown that rivers and streams have greater faecal indicator bacteria (FIB) loadings as they flow through urban areas compared to their upstream agricultural or forested reaches (Goto and Yan, 2011; Paul-Mercado et al., 2016).

Furthermore, a number of studies have identified urban rivers and streams as faecal pollution sources for the bathing areas into which they discharge (Izbicki et al., 2009; Sercu et al., 2009; Bedri et al., 2016). Urban bathing and fresh water systems play important roles as areas of recreation and beauty as well as serving as sources of food and drainage (Hurlimann and Wilson, 2018). Their importance is such that the European Union (EU) has implemented the Bathing Water Directive to safeguard public health. Under this Directive, EU member states are required to identify faecal pollution sources by enumerating FIB (E. coli and intestinal enterococci) that impact their coastal and inland waters (Chave, 2001; WHO, 2003; EU, 2006; Frewtrall and Kay, 2015). Although marine estuaries have received attention in terms of how they are impacted on by ARGs, studies assessing the effects of ARG contamination in public bathing waters remain limited, with ARGs in E. coli being the primary focus of those that have been published (Leonard et al., 2015; Maravic et al., 2015).

Urban streams that have been studied with respect to faecal or ARG pollution have typically been longer than 10 km and, in many cases, receive treated wastewater or flow through agricultural areas (Xu et al., 2016). Therefore, the aim of this study was to determine whether small (<4 km), completely urban streams contain sulphamidine, tetracycline, ciprofloxacin and β-lactam ARGs, which are the most prescribed antibiotic classes in Ireland for community use (ECDC, 2018). Furthermore, we wanted to determine the extent of faecal contamination in these urban streams, whether this contamination is of human origin and if increased ARG levels correlate with levels of faecal indicator markers. Finally, we aimed to determine the extent of human, gull and dog faecal and ARG pollution in the public bathing waters that the studied urban streams directly discharge into.

2. Materials and methods

2.1. Study area and sampling

Dublin, the capital of Ireland, is a coastal city with a population of approximately 560,000 people within its city limits. The River Liffey is the largest river in Dublin which flows through the city centre. As it discharges into Dublin Bay it receives treated effluent from the WWTP at Ringsend (currently operating at a capacity of 1.9 million population equivalents). This WWTP operates a primary settling stage, followed by secondary treatment in 24 sequencing batch reactors. Moreover, during each bathing season (May to September) tertiary treatment is achieved by ultraviolet disinfection. Dublin Bay is a UNESCO biosphere and is home to thousands of protected native and migratory birds that roost on or near the coast.

Several small streams that are completely urban along their courses discharge to Dublin Bay. The Elm Park and Trimleston streams are 3.8 km and 1.7 km long respectively with widths of <3 m and depths typically <10 cm. Both streams flow through urban areas with a population of approximately 40,000 people (which, for this work, we refer to as the catchment area of the streams) before discharging onto two of Dublin’s three designated bathing areas (Sandymount Strand and Merrion Strand) (Fig. 1).

Bi-monthly water samples (n = 156) were collected over a 13-month period from August 2017 to August 2018 at the Elm Park stream and Trimleston stream discharge points as well as from the Liffey Estuary downstream of the Ringsend WWTP discharge point. Furthermore, samples were collected at the Merrion Strand, Sandymount Strand and Dollymount Strand compliance points, the latter does not receive water from an urban stream (Table S1). Using sterile 1 L bottles, duplicate grab samples were collected from the middle of the streams or 2 m from the Liffey Estuary bank, at a depth of 20 cm when possible. Samples were stored at 4 °C before being processed within 6 h.

2.2. Enumeration of faecal indicator bacteria

FIB were enumerated using standard filtration methods (Environment Agency, 2000). Briefly, samples were passed through 0.45 μm nitrocellulose filter membranes (Nalgene, Thermo Scientific). Membranes were incubated on Tryptone Bile X-Glucuronide agar (Sigma-Aldrich) and incubated at 44 °C for 24 h to confirm intestinal enterococci. FIB levels were expressed as CFU/100 ml.

2.3. Extraction of DNA

Water samples (100 ml) were concentrated by filtration through 0.22 μm nitrocellulose filters and subsequently transferred to ice cold lysis buffer (5 M guanidine isothiocyanate, 100 mM EDTA [pH 8.0], 0.5% [w/v] sodium lauryl sarcosinate) and stored at −20 °C until extraction. DNA was extracted using a previously described modification of the DNeasy Blood and Tissue kit protocol (Qiagen) (Gourmelon et al., 2007).
### 2.4. MST and ARG quantification

The *sul1*, *tet(O)*, *qnrS*, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> genes and the HF183, gull and dog MST markers were quantified using SYBR Green I (Roche) on the Roche Lightcycler 96 platform (Roche) (Table 1). The ARG and MST gene levels were expressed as gene copies per 100 ml (gc/100 ml).

All qPCR cycle conditions included a 10 min incubation at 95 °C and a melt curve analysis. Specific primers and amplification conditions were used to amplify each target gene (Table 1). Fragments of each ARG were cloned into the pGEM-T easy plasmid. The HF183, gull and dog MST markers were cloned into the pBluescript plasmid. Plasmid DNA was extracted using the QIAprep spin miniprep kit (Qiagen) and after linearization, standard curves between 10<sup>6</sup> and 10<sup>10</sup> gene copies were included in each run to quantify target gene levels in each sample. The efficiency of each reaction was determined using the E = 10(1/slope)-1 equation (Rutledge and Côté, 2003). The limit of detection was determined as the lowest concentration of DNA detected in 95% or more of replicates and the limit of quantification was determined as the lowest concentration of DNA quantified within 0.5 standard deviations of the log<sub>10</sub> concentration (Table 1) (Rutledge and Stewart, 2008). All standard curves had an R<sup>2</sup> value >0.985.

For each sample, duplicate qPCR reactions were completed from the undiluted sample and a ten-fold diluted sample. Triplicate ‘no-template’ controls were included in all qPCR runs. Each 20 μl reaction mixture contained 10 μl of SYBR Green I Master (Roche), 500 nM of each of the forward and reverse primer and 1 μl of sample.

### 2.5. Statistical analysis

Statistically significant differences between the distributions of concentrations of FIB, microbial source tracking (MST) markers and ARGs were determined using the Kruskal Wallis test with Dunn’s post hoc analysis. Spearman correlation analyses of the levels of FIB, MST markers and ARGs were performed using Prism8 GraphPad software. A significance cut-off of p ≤ 0.05 was used for all analyses.

### 3. Results and discussion

#### 3.1. Urban streams in Dublin contain high levels of FIB and the human MST marker HF183

*E. coli* levels in the Elm Park and Trimleston streams and the Liffey Estuary varied by three orders of magnitude between 2.28 × 10<sup>3</sup> and 2.28 × 10<sup>6</sup> FIB per 100 ml.

#### Table 1

| Target gene Primer sequence | Amplicon size | LoD | LoQ | Cycling condition | Reference |
|-----------------------------|--------------|-----|-----|-------------------|-----------|
| *bla*<sub>TEM</sub> | F: CTATGGGACCCACCAACGATA R: ACCGGCTTCGAGCTGGTTT | 251 bp | 1.7 gc/100 ml | 1.7 gc/100 ml | 40 cycles (95 °C - 10 s, 60 °C - 20 s, 72 - 20 s) | (Sidrach-Cardona et al., 2014) |
| *bla*<sub>SHV</sub> | F: GCCGTTCGACATGACACCATTT R: TTCTTCTGCGGATATGCTTT | 110 bp | 1.2 gc/100 ml | 1.2 gc/100 ml | 40 cycles (95 °C - 15 s, 64 °C - 30 s, 72 - 20 s) | (Xi et al., 2009) |
| *bla*<sub>CTX-M</sub> | F: GCGGCCGCTTACTGACGACG R: CTTATCGGCGCCCTAATGCTC | 103 bp | 1.8 gc/100 ml | 3.5 gc/100 ml | 45 cycles (95 °C - 15 s, 20 °C - 20 s, 72 - 20 s) | (Marti et al., 2014) |
| *qnrS* | F: GACGCTCTAATCTTGTCCG T: GTGCACTTCGTCGCACTT | 119 bp | 1.2 gc/100 ml | 2.4 gc/100 ml | 45 cycles (95 °C - 15 s, 62 °C - 20 s, 72 - 20 s) | (Marti and Balcázar, 2013b) |
| *tet(O)* | F: ACCGARATGTTTAATCTTAC R: TGACGATTACGATGCTGAC | 171 bp | 1.1 gc/100 ml | 2.2 gc/100 ml | 45 cycles (95 °C - 10 s, 50 °C - 15 s, 72 - 15 s) | (Aminov et al., 2001) |
| *sul1* | F: CCACGCGACACAGTCGAC T: TGAGCTCCCAGCATCTTG | 163 bp | 1.2 gc/100 ml | 2.4 gc/100 ml | 50 cycles (95 °C - 15 s, 65 °C - 30 s, 72 - 30 s) | (Pei et al., 2006) |
| HF183 | F: ATCAATGATCTCATACGTCG R: TACCCCGTCTACAGTCTTG | 82 bp | 1.1 gc/100 ml | 2.2 gc/100 ml | 45 cycles (95 °C - 5 s, 60 °C - 15 s, 72 - 20 s) | (Seurinck et al., 2005) |
| Dog Marker | F: GCCGTGATATGACCGTACG R: CAAAGCGGTCTCTCTG | 250 bp | 25 gc/100 ml | 25 gc/100 ml | 40 cycles (95 °C - 15 s, 60 °C - 15 s, 72 - 20 s) | (Dick et al., 2005) |
| Gull Marker | F: TGCAAGGCGACTTTTGAG R: GTCAAAAGGCCAGCATTACTA | 418 bp | 8 gc/100 ml | 8 gc/100 ml | 50 cycles (95 °C - 5 s, 64 °C - 15 s, 72 - 10 s) | (Lu et al., 2008) |
8.7 × 10^4 CFU/100 ml (Fig. 2a). Intestinal enterococci levels at these sites ranged from 10 CFU/100 ml to 7.08 × 10^4 CFU/100 ml (Fig. 2b). The high levels of E. coli and intestinal enterococci found in the urban streams studied here are within the same range as those reported for other sewage impacted streams and rivers (Paule-Mercado et al., 2016). In some instances, the HF183 levels in the urban streams were greater than those in the Liffey Estuary (Fig. 2a). The levels of HF183 quantified in the urban streams and Liffey Estuary are similar to levels quantified in human waste impacted urban estuaries and rivers from other studies (Olds et al., 2018). Raw sewage contains levels of around 1 × 10^8 gc/100 ml of the HF183 MST marker, and so the levels observed in these urban streams can be considered to be high in some instances (Hughes et al., 2017). This provides evidence that these small urban streams are being heavily impacted on by human faecal contamination, which may be due to sewer overflows and misconnections (Ellis and Butler, 2015).

3.2. Urban bathing waters are polluted with faeces of human and animal origin

Levels of E. coli (<10–1.38 × 10^4 CFU/100 ml) and intestinal enterococci (<10–4.33 × 10^3 CFU/100 ml) in the bathing waters were typically two orders of magnitude lower than those in the urban streams and were not significantly different from each other (Kruskal Wallis).
Interestingly, over the sampling period Merrion and Sandymount Strand samples more frequently exceeded the 90th percentile value that defines sufficient water quality in the Revised Bathing Water Directive (500 CFU/100 ml and 185 CFU/100 ml for *E. coli* and intestinal enterococci respectively) than Dollymount Strand (EU, 2006). This may be a result of urban streams discharging directly into Merrion and Sandymount Strand bathing waters but not into Dollymount Strand bathing waters. This observation is in agreement with the results of Aragonés et al. who found urban bathing waters to have significantly higher faecal indicator bacteria levels than semi-urban bathing waters, likely because they are impacted on more frequently by faeces contaminated discharges (Aragonés et al., 2016).

HF183 marker levels in the three bathing waters were not significantly different from each other and were one to two orders of magnitude lower than those in the urban streams (BLD to 2.79 × 10^7 gc/100 ml; Kruskal Wallis, p < 0.05, Fig. 3a). The large range of HF183 levels observed in the studied bathing waters is consistent with reports for other urban stream impacted bathing waters. Seurinck et al. showed that urban beaches in Belgium had levels as high as 1 × 10^6 gc/100 ml and more recently, Molina et al. found that urban creek impacted beaches in the United States had HF183 levels up to 1 × 10^7 gc/100 ml (Seurinck et al., 2006; Molina et al., 2014). As there were no significant differences in HF183 levels between the urban stream impacted beaches (Merrion and Sandymount Strands) and Dollymount Strand; the latter may be impacted by some other source of human faeces not studied here.

Gull and dog microbial source tracking markers were more frequently quantifiable in Sandymount and Merrion Strand bathing waters (~98% of samples) compared to Dollymount Strand (42% of samples). The levels of the gull and dog markers ranged from below the detection limit to 5.37 × 10^4 gc/100 ml and from below the detection limit to 6.98 × 10^4 gc/100 ml, respectively (Fig. 3b). Gulls are known to roost and forage in the shallow waters of Merrion and Sandymount Strand and visual evidence of fouling events is more evident in these waters than in Dollymount Strand. Additionally, these two bathing waters are popular recreational sites for dog walkers. This may explain why these MST markers are more prevalent in Sandymount and Merrion Strand than in Dollymount Strand. Thus, bird and dog fouling events appear as non-point sources of faecal pollution in the bathing waters studied although further research is required to determine their total contribution to the overall pollution levels.

### 3.3. Antibiotic resistance genes are present in urban streams and bathing waters

The ARGs studied in this work were prevalent in the urban streams and Liffey Estuary; indeed *bla*TEM, *qnrS* and *tet*(O) were identified in all of the samples tested. The *bla*TEM, *bla*CTXM and *sul1* ARGs were found in over 88% of the urban stream and Liffey Estuary samples. All of the ARGs studied were more abundant in the Liffey Estuary than in the urban streams. ARG levels were typically between 1 and 2 orders of magnitude higher in urban streams than in bathing waters. *bla*TEM was the only ARG found in all of the samples from the three bathing waters studied, although *tet*(O) was also found in all of the Dollymount and Sandymount Strand samples and in 92% of Merrion Strand samples. *sul1* was found in between 89% and 96% of the bathing water samples and *bla*TEM, *bla*CTXM and *qnrS* were found in between 65% and 89% of these samples.

At all sites *sul1* was the more abundant, by 1 to 2 orders of magnitude, compared to other ARGs (Fig. 4a-d, Kruskal Wallis, p < 0.05), with levels ranging from below the limit of detection to 2.48 × 10^6 gc/100 ml in the Elm Park and Trimleston streams. Other studies have also found *sul1* to be the most abundant ARG in river systems which is, in part at least, likely a result of its association with *int1* type integrons. These integrons are mobile genetic elements that are often associated with conjugative transposons and plasmids that aid in the dissemination of the genes they carry (Xu et al., 2016). Absolute levels of *sul1* were 100-fold lower in the bathing waters (below the limit of detection to 7.99 × 10^4 gc/100 ml) relative to the urban streams. Although sulphonamides represent <10% of antibiotics prescribed for community use in Ireland a number of studies have demonstrated *sul1* as being a
useful marker for human pollution and the high levels of sul1 observed in this study suggests that the sites studied in this work are impacted by human faecal waste (ECDC, 2018; Szekeres et al., 2018; Lye et al., 2019).

The expression of extended spectrum β-lactamases (ESBLs) by pathogens is of particular concern as they confer resistance to clinically important penicillin and cephalosporin antibiotics that are used as first line drugs to treat infections which represent more than 50% of antibiotic prescriptions in Ireland (ECDC, 2018). blaTEM, blaSHV and blaCTX-M levels were generally 10-fold lower in the Elm Park and Trimleston streams compared to those in the Liffey Estuary. blaCTX-M (below the limit of detection to 3.02 × 10⁴ gc/100 ml) concentrations were typically 10-fold lower than blaTEM and blaSHV (6.75 × 10² to 5.65 × 10⁵ gc/100 ml) in the Trimleston, Elm Park streams and the Liffey Estuary. The levels of blaTEM observed in the Liffey Estuary are in the same range as those found in the WWTP impacted Zenne river in Belgium (1 × 10⁵ to 1 × 10⁷ gc/100 ml) by Proia et al. (Proia et al., 2018). Although the overall level of bla genes were 10-fold lower in baptismal waters compared to the urban streams, the same trend was observed with levels of blaCTX-M (BDL to 1.11 × 10^{4} gc/100 ml) 10-fold lower than blaTEM and blaSHV (2.24 × 10² to 1.58 × 10⁵ gc/100 ml). Although, ESBL variants of blaTEM and blaSHV can confer resistance to cephalosporins, non-ESBL variants that may be selected for by penicillins alone also exist, whereas all blaCTX-M genes are ESBL genes. Thus, a greater reliance on penicillins in the Irish community compared to cephalosporins may explain the higher levels of blaTEM and blaSHV compared to blaCTX-M observed in this study.

The qnrS levels quantified in the Liffey Estuary (1.36 × 10⁴ to 2.22 × 10⁵ gc/100 ml) are in the same range as those found by Rodriguez-Mozaz et al. in the WWTP impacted Ter river in Spain (Rodriguez-Mozaz et al., 2015). Interestingly, although qnrS could not be identified in all bathing water samples, when it was, the observed levels were similar to those in the Elm Park and Trimleston streams (BDL to 5.12 × 10⁴ gc/100 ml). This suggests that there may be another source of qnrS on Merion and Sandymount Strands. qnrS may be present in environmental bacterial species in these marine waters; for example Marinobacter spp. and Photobacterium spp. are known to harbour mobile genetic elements encoding qnrS (Canton, 2009; Tomova et al., 2018).

tet(O) was the second most abundant ARG in the urban streams (9.78 × 10² to 1.97 × 10⁶ gc/100 ml) with median levels ranging from 10-fold to 100-fold lower than those in the Liffey Estuary. Previous studies have demonstrated that increased levels of tet(O) are associated with anthropogenic activities. Proia et al. demonstrated that tet(O) levels in the Zenne river in Belgium increased from its agricultural upstream to its urban downstream regions (Proia et al., 2018). Median levels of tet(O) in all the bathing waters examined in this study were 10-fold to 100-fold lower than those of the urban streams. Tetracyclines are still commonly prescribed for community use in Ireland, representing approximately 10% of antibiotic prescriptions which may explain the high concentrations observed here (ECDC, 2018).

Previous studies have demonstrated that rivers have higher concentrations of ARGs after they receive WWTP discharges. As such, the high levels of ARGs found in the Liffey Estuary are likely a result of discharge from the Ringsend WWTP. To the best of our knowledge this study represents the first time small urban streams (~4 km long) have been studied in respect to ARG pollution, particularly in relation to urban bathing waters that they discharge into. The high levels of ARGs observed in the urban streams and bathing waters in this study demonstrates that such waters are a potential source for ARG dissemination.

### 3.4. Do high levels of faecal indicators correlate with increased ARG levels?

To determine if there was a relationship between ARGs and faecal indicators in the estuary, urban stream and bathing waters, a correlation analysis between these variables was carried out (Table 2). Significant correlations (R² = 0.392–0.855, p ≤ 0.005) were observed in the Liffey Estuary between the levels of E. coli, intestinal enterococci and HIFB183 and all of the studied ARGs. This demonstrates that human faecal pollution is associated with increased abundance and enrichment of ARGs in the Liffey Estuary, which is impacted by the Ringsend WWTP. This is in agreement with the recently published report by Karkman et al. which showed that human faecal pollution can explain ARG levels in faecally impacted waters (Karkman et al., 2019). The Ringsend WWTP currently treats faecal waste from the greater Dublin area (approximately 1.9 million people). Given that WWTP are hotspots for ARG dissemination, these observations in the Liffey Estuary are to be expected (Rizzo et al., 2013).

Correlations in the urban streams were less clear as relationships between faecal indicators were observed for some but not all of the ARGS as was the case for the Liffey Estuary. For example, in the Trimleston

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**Table 2**: Correlations Between Faecal Indicators and Antibiotic Resistance Genes. *p ≤ 0.05, **p ≤ 0.005, ***p ≤ 0.001.

| Site          | Faecal indicator and MST markers | blaTEM | blaSHV | blaCTX-M | qnrS | tet(O) | sul1 |
|---------------|---------------------------------|--------|--------|----------|------|--------|------|
| Liffey Estuary| E. coli                         | 0.855***| 0.580**| 0.730***| 0.716***| 0.524* | 0.709***|
|               | IE                              | 0.808***| 0.505* | 0.720***| 0.725***| 0.606**| 0.744***|
|               | Human                           | 0.769***| 0.392* | 0.612** | 0.633** | 0.718***| 0.718***|
| Elm Park Stream| E. coli                         | 0.562*  | 0.409* | 0.168   | 0.330  | 0.224  | 0.233|
|               | IE                              | 0.341   | −0.004 | 0.086   | −0.010 | 0.176  | −0.017|
|               | Human                           | 0.201   | 0.108  | 0.030   | 0.418* | 0.288  | 0.168|
| Trimleston Stream| E. coli                        | 0.321   | 0.489**| 0.270   | 0.042  | 0.642**| 0.189|
|               | IE                              | 0.234   | 0.662***| 0.391   | 0.226  | 0.420**| 0.447|
|               | Human                           | 0.458*  | 0.177  | 0.214   | 0.555**| 0.596* | 0.461*|
| Dollymount Strand| E. coli                        | −0.214  | 0.061  | −0.289  | 0.001  | 0.182  | 0.190|
|               | IE                              | −0.078  | −0.061 | −0.282  | 0.138  | 0.129  | 0.341|
|               | Human                           | 0.535*  | 0.203  | 0.416   | 0.299  | 0.475* | 0.090|
|               | Gill                            | −0.011  | 0.275  | 0.297   | 0.117  | −0.267 | −0.143|
|               | Dog                             | 0.213   | 0.108  | 0.108   | −0.134 | −0.2432| 0.374|
| Sandymount Strand| E. coli                        | 0.285   | −0.384 | −0.091  | −0.183 | 0.458* | 0.115|
|               | IE                              | 0.374   | −0.254 | −0.038  | −0.125 | 0.638**| 0.280|
|               | Human                           | 0.543*  | 0.332  | 0.280   | 0.031  | 0.626**| 0.561*|
|               | Gill                            | 0.413*  | −0.406 | −0.361  | −0.183 | 0.216  | 0.110|
|               | Dog                             | 0.221   | −0.266 | −0.271  | −0.207 | 0.205  | 0.047|
| Merrion Strand| E. coli                         | −0.122  | −0.170 | −0.389  | 0.120  | 0.476* | 0.266|
|               | IE                              | −0.027  | −0.196 | −0.471  | 0.144  | 0.421**| 0.314|
|               | Human                           | 0.295   | −0.374 | −0.327  | 0.196  | 0.371  | 0.310|
|               | Gill                            | −0.097  | −0.409 | −0.097**| −0.200 | 0.417  | 0.083|
|               | Dog                             | −0.259  | 0.247  | −0.084  | −0.061 | 0.299  | −0.209|
Interestingly, tochthonous bacteria may also be contributing to poor correlations. In-between the levels of ARGs and faecal indicators may be a result of the dog fouling events in these bathing waters. Furthermore, no correlations were observed between blaCTX-M and the faecal indicators in either stream. These observations are likely a result of the dynamics of faecal pollution in the urban streams. For instance, catchment size will impact on the relationship between faecal pollution and ARG levels in urban streams (Milledge et al., 2018). The two urban streams studied here flow through a relatively small catchment area of approximately 40,000 people. In a smaller catchment there are fewer individuals shedding ARGs in their stool. Additionally, the urban streams are sporadically impacted on by faecal pollution (Fig. 2a, b). Due to the intermittent nature of faecal discharge in these urban streams, correlations between faecal indicators and ARGs are infrequently observed. Furthermore, the FIB enumerated in this study are not the only bacteria of faecal origin that can harbour ARGs, for example blaTEM and qnrS encoding Klebsiella spp. and tet(O) encoding Clostridium spp. are prevalent in human faeces (Bing et al., 2015; Rowe Taitt et al., 2017). It is likely that E. coli and intestinal enterococci die of at different rates to these ARG encoding bacteria. Thus, in an environment sporadically impacted by high levels of faecal pollution, correlations will be more difficult to identify. Interestingly, more correlations between ARGs and faecal indicators were observed in the Trimleston Stream than in the Elm Park Stream. This may suggest that the Trimleston stream is more consistently impacted on by faecal pollution than the Elm Park Stream. Interestingly, the tet(O) levels significantly correlated with E. coli, intestinal enterococci and HF183 levels in Sandymount Strand (Table 2), which was also observed in the Trimleston Stream. However, in general, ARG levels did not correlate well with E. coli and intestinal enterococci in the urban bathing waters examined. To determine if animal faecal pollution contributes to ARG levels in these bathing waters a correlation analysis was conducted between gull and dog MST markers and the ARGs. Only a single positive correlation was found between blaTEM and the gull faeces MST in the Sandymount Strand bathing water \( (R^2 = 0.413; p < 0.05) \). As discussed in relation to the urban streams, poor correlations between the levels of ARGs and faecal indicators may be a result of the sporadic nature of faecal pollution in these waters. The presence of autochthonous bacteria may also be contributing to poor correlations. Interestingly, tet(O) more frequently correlated with faecal indicators including HF183 in urban streams and bathing waters, indicating that the small urban streams may contribute to the ARG contamination of these bathing waters. The poor correlation between gull and dog faecal pollution and ARGs may result from the sporadic and diffuse nature of these pollutants. However, gull and dog faeces have been shown to frequently harbour ARGs of clinical importance and so fouling events in shallow waters may still pose a health risk in this regard (Argudin et al., 2017; Ahlstrom et al., 2018).

4. Conclusions

WWTPs are known to pollute receiving waters with high levels of faecal indicator bacteria and ARGs. However, very few studies have focused on faecal and ARG pollution in small urban streams not impacted on by treated waste. To the best of our knowledge this is the first study to focus on faecal and ARG pollution in small urban streams (≤4 km in length) and the bathing waters they discharge into. The urban streams in this study discharge high levels of human faecal matter and ARGs into public bathing waters, indicating that small urban streams and the bathing waters they discharge onto may pose a previously unrecognised health risk as well as an environment for horizontal gene transfer of ARGs between pathogens and enteric commensal bacteria. This ARG contamination may be further exacerbated by gull and dog fouling events in these bathing waters. Our work also indicates that the sporadic relationship between faecal indicators and ARGs is likely a result of the infrequent nature of faecal indicator bacteria pollution in these waters results.

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Credit authorship contribution statement

Liam J. Reynolds: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. Laura Sala–Comorera: Investigation, Validation. Niamh A. Martin: Investigation. Tristan M. Nolan: Validation. Jayne H. Stephens: Investigation. Aurora Gitt: Investigation. Gregory M.P. O’Hare: Writing – review & editing. John J. O’Sullivan: Writing – review & editing, Wim G. Meijer: Conceptualization, Supervision, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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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