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Assessing the impact of recreational water use on carriage of antimicrobial resistant organisms

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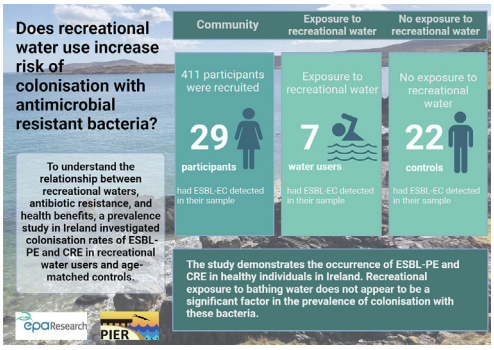
HIGHLIGHTS

- The carriage of AMR Enterobacterales by recreational water users and controls was compared.
- A carriage rate of 7.1 % of ESBL-PE in healthy individuals was observed.
- Water users were less likely than controls to carry ESBL-PE (RR = 0.34, p = 0.007)

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding the role of exposure to natural recreational waters in the acquisition and transmission of antimicrobial resistance (AMR) is an area of increasing interest. A point prevalence study was carried out in the island of Ireland to determine the prevalence of colonisation with extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) and carbapenem-resistant Enterobacterales (CRE) in recreational water users (WU) and matched controls. A total of 411 adult participants (199 WU, 212 controls) submitted at least one faecal sample between September 2020 – October 2021. In total, 80 Enterobacterales were isolated from 73 participants. ESBL-PE were detected in 29 (7.1 %) participants (7 WU, 22 controls), and CRE were detected in nine (2.2 %) participants (4 WU, 5 controls). No carbapenemase-producing Enterobacterales (CPE) were detected. WU were significantly less likely to harbour ESBL-PE than controls (risk ratio = 0.34, 95 % CI 0.148 to 0.776, χ^2 7.37, p = 0.007). This study demonstrates the occurrence of ESBL-PE and CRE in healthy participants in Ireland. Recreational exposure to bathing water in Ireland was associated with a decreased prevalence of colonisation with ESBL-PE and CRE.

1. Introduction

Factors that contribute to the development of antimicrobial resistance (AMR) are intricate and multifaceted (D'Costa et al., 2011). There is

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extensive evidence to link acquired AMR to intensive use and misuse of antimicrobials in human and animal healthcare among other applications (Prestinaci et al., 2015). AMR was long considered primarily a clinical issue, but it is increasingly recognized that the environment plays a significant role in the emergence and spread of AMR (Stanton et al., 2022; Larsson and Flach, 2021; Hooban et al., 2020; Martins and Rabinowitz, 2020; Bevan et al., 2017).

Extended-spectrum beta-lactam antimicrobials, such as third-generation cephalosporins and carbapenems, are categorised as critically important antimicrobials by the World Health Organization (2019). Acquired resistance to these antimicrobial agents is therefore a major concern. The Enterobacterales are an order of bacteria that includes many species, such as *Escherichia coli*, that are naturally present as colonising bacteria in the gastrointestinal tract of humans and animals and are shed in faeces (Kempf et al., 2022; Martinson and Walk, 2020; Tenaillon et al., 2010). They can survive for variable periods in the environment and may transfer to other humans and animals via food and water (Jang et al., 2017). To effectively address the issue of AMR in Enterobacterales, it is imperative to recognise the connections between humans, animals and the environment. A One Health approach that assesses all three components is required to gain a holistic understanding of AMR dissemination (McEwen and Collignon, 2018; Queenan et al., 2016).

Although Enterobacterales are naturally present in the colon, they are also associated with infection at other sites including infection of the urinary tract and bloodstream (Assawatheptawee et al., 2022). Extended-spectrum β-lactamase (ESBL) enzymes mediate resistance to third-generation cephalosporins and are often encoded on mobile genetic elements (MGEs). Resistance mechanisms encoded on MGEs are of particular concern because they readily transfer resistance to other bacteria (Vinayamohan et al., 2022; Sun et al., 2019). Extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-PE), and carbapenemase-producing Enterobacterales (CPE), cause invasive infections and pose a significant clinical challenge globally (Jørgensen et al., 2017b; De Kraker et al., 2013).

Most recent data for Ireland reveals that in 2021, 10.2 % of *E. coli* associated with invasive infection were resistant to third-generation cephalosporins (Health Protection Surveillance Centre, 2022), whilst 73 % of samples from on-farm hatching systems in 2022 were positive for beta-lactam resistant *E. coli* (Byrne et al., 2022) and 30.3 % of samples from fattening pigs in 2019 were ESBL-producing *E. coli* (European Food Safety Authority, (EFSA), 2022). Furthermore, it is apparent from the Irish national inpatient screening programme for colonisation with CPE that this is also a significant issue among people admitted to acute hospitals, with 58 new CPE patients identified in December 2022 (HSE, 2022; Vellinga et al., 2021). There are no published studies regarding the prevalence of colonisation with ESBL-producing Enterobacterales or CRE in otherwise healthy individuals in Ireland.

Engaging with blue spaces and the use of recreational waters has associated benefits for physical and mental health and overall well-being (Britton et al., 2020). However, the presence of microorganisms introduced to these waters by wastewater or runoff from agricultural lands poses a threat to human health (Fewtrell and Kay, 2015). At present, untreated wastewater is discharged directly into waterways at 32 locations in Ireland, with a further 12 locations receiving treated effluent failing to meet the European Union Wastewater Treatment standards (S.I No. 48 of 2010), in 2021 (EPA, 2021a). These 12 locations accounted for 49 % of the total wastewater collected from Ireland's 174 large urban areas, influencing bathing water classifications (EPA, 2021a). Current bathing water quality classifications (Directive 2006/7/EC) rely on the detection of faecal indicator bacteria as a determinant of water quality and risk. These indicators do not address all the organisms of public health concern, for example, viruses or AMR bacteria (Farrell et al., 2021). ESBL-PE and CRE/CPE have been detected in Irish waters, including bathing waters, in recent years (Hooban et al., 2021, 2022; Mahon et al., 2017, 2019), with ESBL-PE and CPE detected in 78 % and 15 % of water samples tested respectively (Hooban et al., 2022). It has been proposed that monitoring for specific AMR organisms, such as EBSL-producing *E. coli* (Hashim et al., 2022; Matheu et al., 2017), should be included as a routine part of bathing water testing in the revision of the EU bathing water directive, which is currently ongoing (European Commission, 2022).

There is a need to better understand the potential role recreational waters play in the transmission and persistence of AMR in the context of the benefits to health associated with blue spaces (Duane and Brychkov, 2023; Britton et al., 2020). A cross-sectional epidemiological study by Leonard et al. (2018a), compared UK surfers with non-bathers, reporting that surfers were four times as likely to carry $bla_{\text{CTX-M}}$ producing *E. coli* (6.3 %) when compared with the control group (1.5 %), (risk ratio = 4.09). A French study reported that an *IMI*-2-producing *Enterobacter asburiae* resulting in bacteraemia was acquired following river exposure, and an identical strain was found from a river sample taken one month later near the exposure region (Laurens et al., 2018).

This study aimed to assess whether there is an association between exposure to natural recreational waters and gut colonisation with AMR Enterobacterales, specifically ESBL-PE and CRE. Secondary objectives were to examine potential risk factors associated with gut colonisation in water users, namely a) whether there was an increase in the prevalence of colonisation with ESBL or CRE in study participants during the bathing season, when more frequent exposure would be expected (Mughini-Gras et al., 2021) and b) whether the microbiological water quality to which bathers were exposed was associated with colonisation with ESBL-PE or CRE.

2. Methods

2.1. Study population and design

Between March 2020 and October 2021, a cross-sectional point prevalence study was conducted on adult volunteers living in the island of Ireland. Participants were segregated into two categories based on their self-reported exposure to natural recreational waters, including rivers, lakes and coastal waters. For the purposes of this study, recreational water users (WU) were defined as those who use natural waters at least three times per month for recreational activities that involve immersion of the participant's head in the water. The control group were defined as those who do not regularly engage in recreational water activities (once per month or less). In so far as practical, controls were matched by age, gender and location (county). Participants were excluded from the study if they reported any one of the following conditions within the previous six months: (a) had taken antibiotics; (b) if they were hospitalised; (c) travelled to a country defined by the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net) as endemic for CPE (Brolund et al., 2019); (d) lived outside of Ireland or; (e) been swimming in natural waters outside of Ireland. Participants were also ineligible if (f) they were under 18 years old; (g) they had a long-term health condition that required frequent hospital visits or increased their susceptibility to infections; (h) they were living with another participant; or (i) their reported recent water exposure did not match the eligibility criteria for either category (water user or control). Eligibility criteria were re-checked upon sample collection, and ineligible participants removed from the study. For the purposes of this study, “healthy” participants were defined as those who had not used antimicrobials in the previous six months, were not recently or regularly hospitalised, and did not suffer from a long-term health condition that would require frequent hospital visits or increase their susceptibility to infections. In order to detect a 10 % difference in the prevalence of gut colonisation with antimicrobial-resistant bacteria (ARB) with 90 % power at a 5 % significance level (2-tailed), it was estimated that a minimum of 145 WU and 145 controls would be required. This calculation assumed a baseline carriage of 1.5 % as reported by Leonard et al. (2018b).

One of the secondary objectives was to examine whether there was an increase in the prevalence of colonisation with ESBL-PE or CPE in study participants during the bathing season (1st June to 15th September), when more frequent exposure would be expected. To investigate this, a subset of participants who had already submitted samples during the non-

bathing period (16th September 2021– 31st May 2022) were invited to submit a second sample during the bathing period (1st June– 15th September 2022). Data from the second samples were analysed separately and only analysed to address this objective.

Ethical approval was obtained from the University of Galway Research Ethics Committee prior to commencing the study (reference number 19-Dec-02).

2.2. Participant recruitment

Participants were initially recruited through in-person engagement with potential participants at recreational water locations. However, following the implementation of COVID-19 restrictions in April 2020, the recruitment strategy was revised and pivoted to traditional and social media campaigns, which were launched in August 2020 and continued until October 2021. Those interested in participating in the study initially registered their interest via an online platform, Qualtrics™. Eligibility checks and informed consent were subsequently completed by phone call.

2.3. Sample and data collection

Participants were sent study kits which included an information sheet, consent form, faecal sample collection instructions, sample collection pot (LIP Diagnostics, Ireland), pre-paid addressed envelope for return and a survey. Participants returned a self-collected faecal sample, signed consent form and completed the study survey. The survey (Supplementary material Form 1) gathered demographic data, alongside exposures to recreational waters and potential risk factors associated with gut colonisation with AMR bacteria (e.g., diet, occupational exposure) and locations of beaches they regularly utilised. Each participant was assigned a unique identification number upon enrolment to the study so that anonymity could be protected as well as blinding the researcher performing analysis on the samples to the exposure status of participants.

Data acquired from completed surveys and laboratory results were tabulated in Microsoft Excel and then imported into IBM SPSS Statistics 27 for analysis. To assess whether the microbiological quality of bathing waters to which water users were exposed is associated with faecal carriage of AMR bacteria, we extracted publically available bathing water quality classification results (from www.beaches.ie) and matched the results to the beaches identified by WU in their completed surveys.

2.4. Faecal sample processing

Samples were tested for resistant Enterobacterales according to the previously developed and validated (unpublished data) protocols of the National Carbapenemase Producing Enterobacterales Reference Laboratory Service, Ireland (NCPERLS). Samples were directly cultured upon arrival on Brilliance™ ESBL-PE agar (Oxoid, sensitivity 95 %, selectivity 94 %) and CHROMagar mSuperCARBA™ (CHROMagar, sensitivity 100 %, specificity 100 %). MacConkey agar was used as a positive control for the growth of viable enteric bacteria. Faecal samples were inoculated directly onto these agars using a cotton swab. Plates were incubated at 37 °C for 36–48 h and inspected for growth of characteristic colonies in accordance with the manufacturer's instructions. One colony of each unique morphological appearance was selected for further analysis. Suspect ESBL-PE and CRE were identified using matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry in accordance with the manufacturer's instructions (Brukner Microflex; MBT Compass 4).

2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed on isolated Enterobacterales by disc diffusion in accordance with EUCAST criteria, and CLSI breakpoints where no EUCAST breakpoints were available. The following antimicrobial agents were tested (Oxoid); ampicillin (10 μg), cefoxitin (30 μg), ceftazidime (10 μg), cefotaxime (5 μg), ertapenem (10 μg), meropenem (10 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg) and trimethoprim (5 μg). ESBL-PE production was assessed using cefpodoxime (10 μg) alone and in combination with clavulanic acid (10 μg/1 μg). Isolates resistant to cefoxitin were considered potential AmpC-producing isolates and tested for AmpC production using combination discs (D68C, MASTDISCS™). The MASTDISCS™ ESBL-PE Detection kit (D66C) were used in accordance with the manufacturer's instructions. Isolates were considered multi-drug resistant (MDR) if the isolate was resistant to one or more agents in at least three antimicrobial classes (Health Service Executive, 2012). *Klebsiella pneumoniae* NTCC 700603 and *Escherichia coli* ATCC 25922 were used as quality control strains.

2.6. Real-time polymerase chain reaction

DNA was extracted using a boil lysis method for real-time PCR, with primer, probe sequences and controls used as outlined in Supplementary Table 1. All ESBL-PE were examined for $bla_{\text{CTX-M-group1}}$, $bla_{\text{CTX-M-group-2}}$, and $bla_{\text{CTX-M-group-9}}$ as previously described (Birkett et al., 2007). Isolates resistant to ertapenem or meropenem were tested for the presence of bla_{VIM}, bla_{IMP} (Mahon et al., 2017), bla_{NDM} (Poirel et al., 2011), $bla_{\text{OXA-48}}$, and bla_{KPC} (Swayne et al., 2011).

2.7. Whole genome sequencing

All ESBL-producing (n = 29), and carbapenem-resistant Enterobacterales (n = 9), were selected for whole genome sequencing (WGS) based on phenotypic confirmation. Genomic DNA was extracted and purified using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's protocol. DNA was quantified using the Qubit dsDNA HS assay kit (Qiagen) and quality assessment was carried out by absorbance ratios using DeNovix DS-11 and Jenway Genova Nano 737501 spectrophotometers. A concentration of >20 μg/mL and an optical density of 1.8–2.0 at 260/280 nm were included as quality control thresholds. Short-read WGS was performed in the Oxford Genomics Centre using a High Multiplex Whole Genome library preparation on Illumina NovaSeq 6000 platform (PE150).

2.8. Bioinformatics analysis

Raw reads were trimmed with Trimmomatic (V0.40), (Bolger et al., 2014), and assembled with Shovill (https://github.com/tseemann/shovill), incorporating SPAdes (Bankevich et al., 2012) alongside quality control assessment using QUAST v4.6.0 (Gurevich et al., 2013). Taxonomic identification was performed using kmers by Kraken 2 (Wood et al., 2019), with sequence types identified using mlst (https://github.com/tseemann/mlst). The programme ABRicate v1.0.1 was used to identify AMR genes employing ResFinder v4.1. (Zankari et al., 2012), and CARD v3.2.4. (McArthur et al., 2013), databases.

PlasmidFinder (v2.1) was used to identify potential plasmids (Carattoli et al., 2014), and Platon v1.6. (https://github.com/oschwengers/platon) used to predict and differentiate plasmid and chromosomal contigs based on protein marker sequences (Schwengers et al., 2020). Assemblies generated with Platon were screened for respective AMR genes by ResFinder, to predict the location of genes as plasmid-bound or chromosomal.

Sequencing reads were also uploaded to the National Center of Biotechnology Information (NCBI) bank, project accession PRJNA942112.

2.9. Statistical analysis

To assess whether there is an association between colonisation and recreational water use, WU were compared to controls as a primary comparison. Participant characteristics were summarised descriptively and the relative risk for the presence of ESBL-PE in faeces was determined with 95 % confidence intervals reported. The analysis used to compare the WU and control groups consisted of pooled logistic regression analysis.

To address the secondary objectives, potential risk factors, such as exposure to poor microbial water quality were summarised descriptively. Differences between groups were assessed using chi-square with a threshold for significant differences set at $P < 0.05$. Data management and analysis were performed using IBM SPSS Statistics 27.

3. Results

3.1. Descriptive characteristics of the included study population

A total of 428 participants from 28 of the 32 counties across the island of Ireland returned complete packs (surveys and samples). Overall, 310 packs were returned by the end of the first sampling period (August 2020–April 2021) and an additional 254 packs were returned by the end of the second sampling period (May–September 2021). The 254 packs returned in the second period included 136 repeat samples and 118 samples from new participants. Seventeen individuals were excluded based on travel (3), exposure to recreational waters outside of Ireland (10) and insufficient sample quantity provided for laboratory analysis (4), leaving 411 participants included in analyses. These comprised 199 WU and 212 Controls. Participant characteristics are presented in Table 1. The majority of participants identified as white Irish ethnicity (91 %) and female (60.3 %). Most ($n = 200$, 48.7 %) were in the age range of 35–54.

3.2. Antimicrobial susceptibility testing

A total of 80 Enterobacterales were isolated from 27 WU ($n = 30$ isolates) and 46 controls ($n = 50$ isolates). Species identified included *E. coli* ($n = 62$, 77.5 %), *Citrobacter freundii* complex ($n = 8$, 10 %), *Enterobacter cloacae* complex ($n = 3$), *Klebsiella* spp. (n = 3), *Hafnia alvei* (n = 2) and one each of *Serratia fonticola*, and *Raoultella ornitholytica*. AST revealed that 42 isolates (52.5 %) were multidrug-resistant (MDR). Nine isolates were resistant to ertapenem (11.25 %) but all were susceptible to meropenem (Fig. 1).

3.3. Faecal carriage of ESBL-PE and CRE

Out of the total participants, 7.1 % ($n = 29$) had ESBL-PE, and 2.2 % had CRE ($n = 9$) isolates. The highest ESBL-PE prevalence was in participants aged between 55 and 64 (8 %) and was slightly higher in women (8 % (20/249) compared to 6 % (9/161) for men) as seen in Supplementary Table 2. The prevalence of ESBL-PE and CRE carriage in WU and controls respectively was 3.5 % ($n = 7$), and 10.4 % ($n = 22$), for ESBL-PE and 2 % (n = 4), and 2.4 % ($n = 5$), for CRE. WU was associated with a lower risk of ESBL-PE colonisation compared to controls (risk ratio = 0.34, 95 % confidence intervals [CI] 0.148 to 0.776, χ^2 7.37, $p = 0.007$). This association was even stronger when WU were compared to control participants who reported no exposure to recreational waters in the past year ($n = 120$) (risk ratio = 0.24, CI 0.106–0.581, χ^2 12.2, $p = 0.0005$). There were no risk factors (e.g. occupational) identified associated with an increased risk of colonisation in either group (Table 1). The majority of control isolates (16/18) were positive for $bla_{\text{CTX-M-group-1}}$ whereas the majority of WU isolates contained a $bla_{\text{CTX-M-group-9}}$ gene (3/5).

3.4. Carriage of ESBL-PE and association with recreational water quality

In total, 76.4 % ($n = 151$) of WU reported engaging in recreational activities in monitored bathing waters, with 96 % ($n = 145$) of them using recreational waters that received an annual water quality classification of "excellent". Of the seven WU harbouring ESBL-PE, all reported recreational activities in monitored bathing waters. Five ESBL-PE WERE detected in WU samples during the first sampling period outside of the bathing season and therefore outside of the time for which water quality test results for those waters were available, however, all seven locations received an annual classification of excellent.

Table 1
Characteristics and potential risk factors of recruited participants in both categories.

Characteristics	WU = 199	Controls = 212	Total = 411
	N (%)	N (%)	N (%)
Gender			
Male	79 (39.7)	82 (38.7)	161 (39.2)
Female	119 (59.8)	130 (61.3)	249 (60.6)
Not recorded	1 (0.5)		1 (0.2)
Age			
18–24	4 (2)	9 (4.2)	13 (3.2)
25–34	26 (13.1)	35 (16.5)	61 (14.8)
35–44	51 (25.6)	57 (26.9)	108 (26.3)
45–54	60 (30.2)	52 (24.5)	112 (27.3)
55–64	44 (22.1)	43 (20.3)	87 (21.2)
65+	13 (6.5)	16 (7.5)	29 (7.1)
Not recorded	1 (0.5)		1 (0.2)
Province of residence[a]			
Leinster	79 (39.7)	99 (46.7)	178 (43.3)
Munster	37 (18.6)	35 (16.5)	72 (17.5)
Connacht	65 (32.7)	69 (32.5)	134 (32.6)
Ulster	11 (5.5)	8 (3.8)	19 (4.6)
Not recorded	7 (3.5)	3 (1.4)	10 (2.4)
Education			
No formal education/training	0	0	0
Primary School education	0	0	0
Secondary education	4 (2)	14 (6.6)	18 (4.4)
Technical or vocational	5 (2.5)	3 (1.4)	8 (1.9)
National certificate or diploma	29 (14.6)	31 (14.6)	60 (14.6)
Third level primary degree (Bachelor)	72 (36.1)	76 (35.8)	148 (36)
Postgraduate diploma or degree (Masters or PhD)	85 (42.7)	88 (41.5)	173 (42.1)
Not recorded	4 (2)		4 (1)
Last time participant was directly exposed to natural recreational waters in Ireland			
<7 days ago	171 (85.9)	2 (0.9)	173 (42.1)
In the past month	14 (7)	12 (5.7)	26 (6.3)
More than a month ago	14 (7)	78 (36.8)	92 (22.4)
More than a year ago	0	120 (56.7)	120 (29.2)
Domestic risk			
Pet or domestic animal in household	108 (54.3)	122 (57.5)	230 (55.9)
Healthcare worker in household	31 (15.6)	33 (15.6)	64 (15.6)
Animal worker in household	11 (5.5)	26 (12.3)	37 (9)
Hospitalisation in household	15 (7.5)	11 (5.2)	26 (6.3)
Antibiotics taken by household member	29 (14.6)	41 (19.3)	70 (17)
Travel by household member in past 6 months	6 (3)	25 (11.8)	31 (7.5)
Occupational risk			
Healthcare work setting	21 (10.6)	32 (15.1)	53 (12.9)
Contact with animals	10 (5)	10 (4.7)	20 (4.9)

[a] Provinces are historical groupings of counties by region as follows Leinster (East), Munster (South and Southwest), Connacht (West) and Ulster (North, including Northern Ireland and three other adjoining counties).

Overall, the majority of WU were categorised as swimmers (98.5 %) (Table 2). No significant differences were detected in the reported intensity or duration of recreational water use among the WU surveyed. A total of 42.4 % ($n = 29$) surfers indicated they "always" or "often" swallowed water when using recreational waters, compared to 31.3 % of body boarders and 14.8 % of swimmers. Of the seven WU with ESBL-EC, all indicated their main recreational activity as swimming.

3.5. In silico analysis of sequenced isolates

In silico MLST using Kraken2 was used to identify species as *E. coli* (n = 30), *Citrobacter freundii* complex (n = 5), *Serratia fonticola* (n = 1) and *Enterobacter cloacae* complex ($n = 1$) (Table 3), concurring with previous identification by MALDI-TOF. MLST of the 30 *E. coli* led to the identification of 13 STs, including ST131 (n = 6), ST69 ($n = 5$), ST10 (n = 4), ST14 ($n = 3$), and ST38 (n = 2). Two novel STs were identified, alongside eight other unique STs.

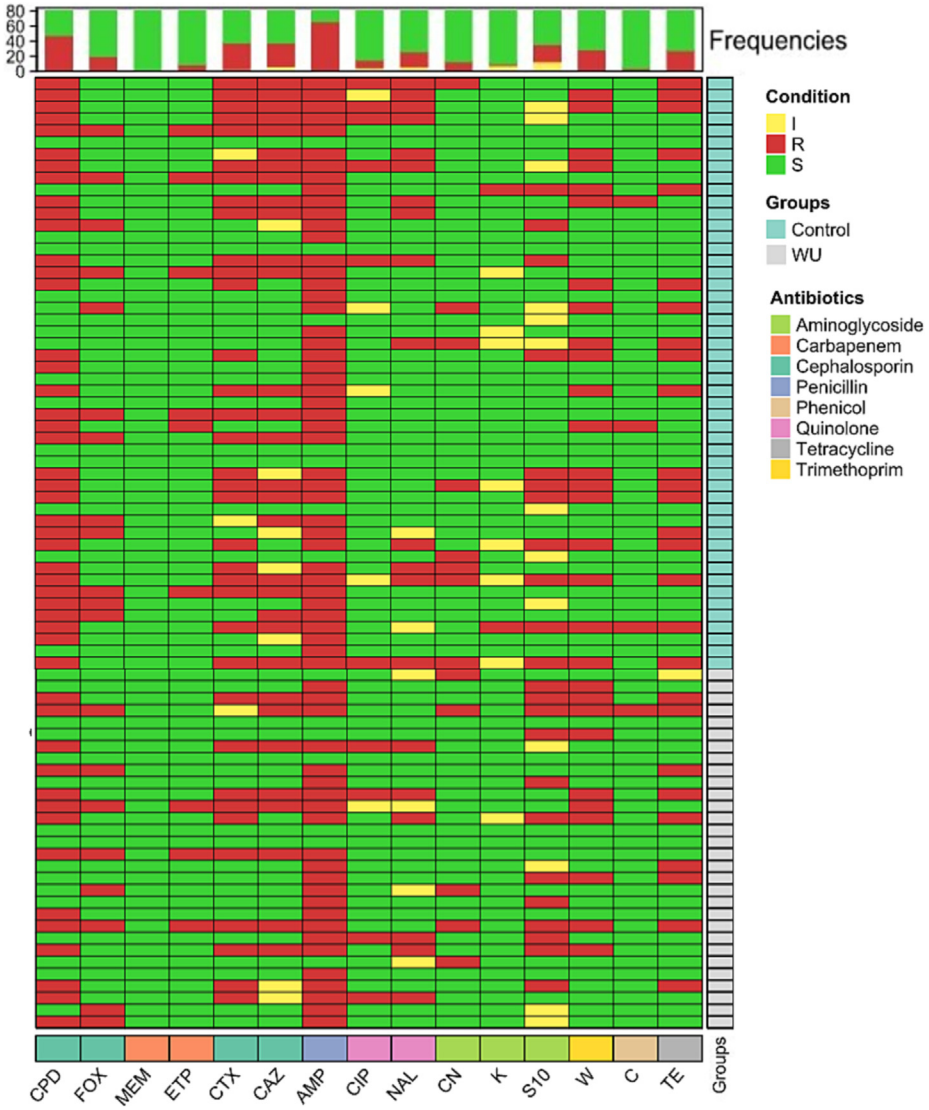

Fig. 1. Heatmap of isolates from WU (n = 43) and Controls (n = 67) that demonstrated non-susceptibility patterns to a panel of 15 antimicrobials. The stacked bar chart on top displays the frequency of resistance occurrence for each of the antimicrobials. Red indicates resistance (R), yellow indicates intermediate (I), and green indicates susceptibility (S). CPD = Cefpodoxime, FOX = Cefoxitin, MEM = Meropenem, ETP = Ertapenem, CTX = Cefotaxime, CAZ = Ceftazidime, AMP = Ampicillin, CIP = Ciprofloxacin, NAL = Naladixic acid, CN = Gentamicin, K = Kanamycin, S10 = Streptomycin, W = Trimethoprim, C = Chloramphenicol, TE = Tetracycline. The graph was plotted in R studio using ComplexHeatmap Package (Gu et al., 2016).

Resistome analysis correlated with the observed phenotypic antimicrobial susceptibility profiles. A total of 230 AMR genes were detected, with those conferring resistance to beta-lactams as the most common genes detected (n = 56). Overall, a median of six resistance genes was identified per isolate. Genetic determinants conferring resistance to the following antimicrobials were identified: aminoglycosides (n = 11 gene types), sulphonamide (n = 4 gene types), trimethoprim (n = 5 gene types), tetracycline (n = 4 gene types), quinolone (n = 4 gene types), phenicols (n = 4 gene types), and fosfomycin (n = 4 gene types).

Among the β-lactam genes detected (n = 55), the $bla_{\text{CTX-M}}$ genes predominated, with 23/38 strains carrying one of the following genes: $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-27}}$, and $bla_{\text{CTX-M-55}}$. Similar to previous studies, $bla_{\text{CTX-M-15}}$ was the most common $bla_{\text{CTX-M}}$ gene (14/23, 65.6 %). Additional ESBL variants included $bla_{\text{SHV-12}}$ (n = 4), $bla_{\text{TEM-1B}}$ (n = 16), $bla_{\text{TEM-210}}$ (n = 1), and $bla_{\text{TEM-141}}$ (n = 1). Whilst $bla_{\text{CTX-M-15}}$ was commonly associated with ST131 (n = 3), this gene was also prevalent in other human infection-associated STs such as ST38 (n = 3) and ST69 (n = 2). No carbapenemase genes were detected, and all CRE isolates harboured AmpC genes, namely bla_{CMY} (n = 6), bla_{DHA} (n = 2) and bla_{ACT} (n = 1).

Plasmid replicons were detected in 35/38 (92.1 %) sequenced isolates. A total of 137 replicons were detected, with the most prevalent being Col-type replicons (n = 49), followed by incompatibility (IncF) (n = 26) as seen in Fig. 2. A median of four plasmid replicons were detected per isolate, with several isolates harbouring up to eight replicons (n = 3), and eight harbouring six replicons each. Chromosomal $bla_{\text{CTX-M}}$ genes were identified in silico in seven isolates, belonging to ST 131 (n = 3), ST28 (n = 2) and two other unique STs, as seen in Fig. 2. The remaining 16 $bla_{\text{CTX-M}}$ genes were predicted plasmid-borne by Platon.

3.6. Variation in the carriage of ARB detection across sampling periods

To investigate variation in the carriage of ESBL across the sampling periods, 136 individuals (72 WU, 68 controls) submitted a sample during the non-bathing season and bathing season. Five participants carried ESBL-PE in both sampling periods (1 WU, 4 Controls). There were six new detections of ESBL-PE in the bathing season (4 WU, 2 Controls). During the non-bathing season, two individuals from each group carried ESBL-PE, which was not detected during the bathing season. The incidence risk ratio

Table 2
ESBL carriage per water activity identified by participants in surveys. Significant results ($p < 0.05$) are indicated by an asterisk and indicate the ESBL-PE carriage when compared with the control group.

Activity	Colonised with ESBL (%)	ESBL-PE not detected (%)	Total	*p*-value	Risk	CI
Surfing	3 (5.6)	51 (94.4)	54	0.278	0.535	0.166–1.723
Body Boarding	1 (3.1)	30 (93.8)	32	0.204	0.301	0.043–2.225
Stand-up paddle boarding or paddle boarding	4 (8.7)	42 (91.3)	46	0.731	0.838	0.303–2.316
Windsurfing	0	9 (100)	9	–	–	–
Swimming	7 (3.6)	185 (94.4)	196	0.009*	0.351	0.154–0.804
Snorkeling	1 (3.1)	31 (96.9)	32	0.191	0.301	0.042–2.158
Scuba	0 (0)	4 (100)	4	–	–	–
Diving	0 (0)	36 (100)	36	–	–	–
Canoeing	2 (3.5)	55 (96.5)	57	0.106	0.338	0.082–1.396

between WU and controls was 1.89 (95 % CI 0.36–9.98, $p = 0.45$). The four controls with ESBL-PE in both sampling points were colonised by ESBL-PE belonging to the same ST, with similar resistomes, consistent with the persistence of the same strain in the gut microbiome. However, two distinct ESBL-producing *E. coli* were detected in one WU on the first and second sampling occasions. In the first sample, a $bla_{\text{CTX-M -14}}$ ST10 *E. coli* was detected, and in the second sample a $bla_{\text{CTX-M -15}}$ ST131-*E. coli* isolate was detected.

4. Discussion

Clinically important AMR bacteria, such as ESBL-PE, and CRE, pose a serious public health threat globally. Our study describes the first large-scale study of ESBL-PE and CRE carriage in water users and people who do not participate in recreational water activities in Ireland. A key knowledge gap in understanding and managing the spread of AMR is investigating the complex role the aquatic environment, such as recreational water, has in the transmission of AMR bacteria (Gross et al., 2022). Identification of ESBL-PE and CRE/CPE organisms in Irish recreational waters (Hooban et al., 2021, 2022; Mahon et al., 2017, 2019), and in other countries (Leonard et al., 2018a; Jørgensen et al., 2017a; Blaak et al., 2014), is of increasing interest as water users may acquire treatment-resistant infections from bathing in natural waters (Leonard et al., 2022). In this study, we found that participants who reported being frequent water users were significantly less likely to be colonised with an ESBL-PE compared to the control group WU (risk ratio = 0.34, 0.148 to 0.776, $\chi 2$ 7.37, $p = 0.007$). These results are in contrast to a recent UK study, which found that frequent surfers were three times as likely as non-bathers to be colonised with third-generation cephalosporin-resistant *E. coli* (Leonard et al., 2018a). These differences might be attributable to different study populations, varying water qualities to which participants may have been exposed, and different analytical methods used to detect ESBL-PE. Leonard et al. (2018a) recruited frequent surfers, who ingest more water during surf sessions compared to other water user groups (Leonard et al., 2015; Stone et al., 2008). In the present study, various types of WU (primarily sea swimmers) were

Table 3
Characteristics of sequenced isolates including sequence types, beta-lactamase genes and plasmid replicons detected. Chromosomally located genes determined by platon and Resfinder are identified by an asterisk.

ID	Category	Species	ST	Beta-lactamase genes	Plasmid replicons
MEH633	Control	*E. hormaechei*	–	$bla_{\text{ACT-14}}$*	ND
MCF1305	WU	*Citrobacter braakii*	–	$bla_{\text{CMY-74}}$*	ND
MEC813	Control	*E. coli*	–	$bla_{\text{CTX-M-15}}$	Col156, IncFIB, IncFIC
MSF1196A	Control	*S. fonticola*	–	$bla_{\text{FONA-6}}$*	Col1, IncFIB, IncFIC
MEC2701	Control	*E. coli*	–	$bla_{\text{CTX-M-15}}$	ColRNAI
MEC724A	WU	*E. coli*	10	$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1B}}$	Col(MG828), ColRNAI, IncB/O/K/Z, IncFIA, IncFIB, IncFIC
MEC2902	Control	*E. coli*	10	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1B}}$	IncFIB, IncFIC, IncI1
MEC1047	WU	*E. coli*	10	$bla_{\text{SHV-12}}$*	ColRNAI, IncFIC
MEC2637	Control	*E. coli*	10	$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1B}}$	Col(MG828), ColRNAI, IncB/O/K/Z, IncFIA, IncFIB, IncFIC
MEC2479	Control	*E. coli*	14	$bla_{\text{TEM-1B}}$ * $bla_{\text{SHV-12}}$	Col156, ColRNAI, IncFIA, IncFIB, IncFIC, IncFII
MEC2831	Control	*E. coli*	14	$bla_{\text{TEM-1B}}$* $bla_{\text{SHV-12}}$	Col156, ColRNAI, IncFIA, IncFIB, IncFIC, IncFII
MEC1940	WU	*E. coli*	14	$bla_{\text{TEM-1B}}$*, $bla_{\text{SHV-12}}$*	Col156, ColRNAI, IncFIA, IncFIB, IncFIC, IncFII
MEC6749	Control	*E. coli*	38	$bla_{\text{CTX-M-15}}$*, $bla_{\text{TEM-1B}}$	Col156, IncFIB, IncFIC
MEC2595A	Control	*E. coli*	38	$bla_{\text{CTX-M-15}}$*	Col156, IncFIB, p0111
MCF2589	Control	*Citrobacter freundii*	62	$bla_{\text{CMY-150}}$*	IncFIA
MEC1043	WU	*E. coli*	69	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$	Col(MG828), Col156, Col8282, ColRNAI, IncB/O/K/Z, IncFIA, IncFIB, IncFIC
MEC1862	WU	*E. coli*	69	$bla_{\text{DHA-1}}$, $bla_{\text{TEM-1B}}$	Col156, Col8282 ColRNAI, IncFIA, IncFIB, IncFIC, IncX1, IncY
MEC2233	Control	*E. coli*	69	$bla_{\text{CMY-138}}$, $bla_{\text{TEM-1B}}$	Col156, Col8282, IncB/O/K/Z, IncFIB, IncFII, IncX1
MEC2078	Control	*E. coli*	69	$bla_{\text{CTX-M-1}}$, $bla_{\text{TEM-1B}}$	IncFIB, IncFII
MEC2674	Control	*E. coli*	69	$bla_{\text{CTX-M-15}}$	Col156, Col8282, IncFIA, IncFIB, IncFII, IncX1
MEC760	Control	*E. coli*	101	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-141}}$	Col(MG828), IncFIB, IncFIC, IncFII, IncFII, IncI1
MEC2840A	Control	*E. coli*	127	$bla_{\text{CTX-M-15}}$*	Col156, IncB/O/K/Z, IncFIB, IncFII
MEC1502A	Control	*E. coli*	131	$bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$	Col(BS512), IncFIA, IncFIB, IncFIC
MEC1601	Control	*E. coli*	131	$bla_{\text{CTX-M-15}}$*, $bla_{\text{TEM-1B}}$	Col156, ColRNAI, IncFIB, IncFII
MEC2088	Control	*E. coli*	131	$bla_{\text{CTX-M-15}}$	Col156, IncFIA, IncFII
MEC681	WU	*E. coli*	131	$bla_{\text{CTX-M-27}}$	Col(MG828), Col156, IncFIA, IncFIB, IncFII
MEC2269	WU	*E. coli*	131	$bla_{\text{CTX-M-14}}$*, $bla_{\text{TEM-1B}}$	Col1, IncFIB, IncFII
MEC1107	Control	*E. coli*	131	$bla_{\text{CTX-M-27}}$*, $bla_{\text{TEM-1B}}$	Col1, IncFIB, IncFII
MEC883	Control	*E. coli*	141	$bla_{\text{CTX-M-1}}$, $bla_{\text{TEM-210}}$	IncFIB, IncFIC, IncI1
MEC2196	Control	*E. coli*	212	$bla_{\text{CTX-M-15}}$	Col440I, IncFIB, IncI1
MCF2205	Control	*C. youngae*	243	$bla_{\text{CMY-53}}$*	Col440II, ColRNAI, RepA
MEC1622	Control	*E. coli*	357	$bla_{\text{DHA-1}}$	Col1, Col1, ColRNAI, IncFII
MCF2874	WU	*C. murliniae*	496	$bla_{\text{CMY-104}}$*	RepA
MCF2197	Control	*C. portucalensis*	728	$bla_{\text{CMY-39}}$*	Col440I
MEC2488	Control	*E. coli*	2852	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$	IncY
MEC2457	Control	*E. coli*	6438	$bla_{\text{CTX-M-15}}$*	Col440I, Col12, ColRNAI, ColpVC, IncB/O/K/Z, IncFIB, IncFIC, IncI1
MEC2539	WU	*E. coli*	11226	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$	ColpVC, IncFIB (2)

ND indicates Not Detected. - depicts a novel ST.

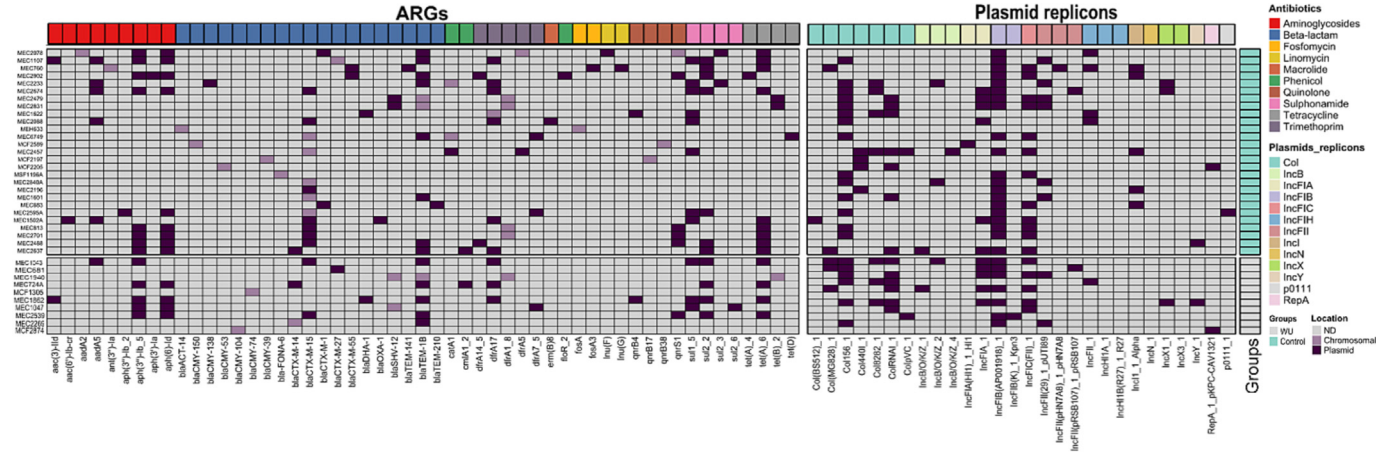

Fig. 2. Heatmap depicting AMR genes detected by ResFinder (left) and plasmid replicons (right) detected and probable locations within the genome. The coloured bars on the top of the ARG section (left) indicate the antimicrobial class the gene confers resistance to, whereas the coloured bars on top of the plasmid replicon section indicate the replicon class. The genomic location of resistance genes are indicated as a plasmid (dark blue), chromosomal (light blue) or absent/Not detected (ND, grey). Heatmap was created in R studio using ComplexHeatmaps (Gu et al., 2016).

recruited, who may experience fewer unexpected head immersions and ingest smaller volumes of water per session compared to surfers (Leonard et al., 2018b). A higher proportion of people engaging in water sports involving boards (e.g. surfing) in this study harboured ESBL-PE than other popular water activities, such as swimming (Table 2). This may be due to the greater frequency of ingesting water reported by these participants, however, the sample size meant statistical significance could not be established.

Moreover, bathing water quality in recent years has improved across Europe (European Environment Agency, 2022). Differences in bathing water quality by time and place may account in part for the differences observed. In 2021, 115 (77.7 %) of Ireland's bathing waters met excellent standards (Environmental Protection Agency, 2021a), compared to 75 % in 2020, giving an average of 76.4 % over the two study years 2020–2021. Of the UK bathing waters classified in the 2015 study period (n = 633), 59.6 % were classified as excellent (European Environment Agency, 2015), in comparison to 76.4 % in the present (2020 − 2021) study sampling period in Ireland (Environmental Protection Agency, 2020). Additionally, Leonard et al. (2018a) used an ampicillin-amended broth to enrich for beta-lactam-resistant and carbapenem-resistant organisms in faecal swabs, before culturing isolates on agar supplemented with cefotaxime. In the current study, faecal samples were cultured directly onto selective screening agar. Nevertheless, the method used allowed us to observe a colonisation rate of 7.1 % across the whole study population for ESBL-EC, which is comparable to similar studies elsewhere in Western Europe where ESBL carriage was observed at 6.1 %, 9 %, 8.6 % and 11.3 % in Denmark, London, Amsterdam and Birmingham, respectively (Arcilla et al., 2020; Otter et al., 2019; Reuland et al., 2016; Wickramasinghe et al., 2012).

Whole genome sequencing (WGS) analysis of the resistant bacteria isolated from participants allowed in-depth characterisation of the strains and resistance genes in colonised individuals. We identified the pathogenic clone *E. coli* ST131 in seven participants (3 WU, 4 Controls). ST131 is well recognized for its successful person-to-person transmission (Torres et al., 2018), and was the most common ST in this study, closely followed by four detections of ST69 (1 WU, 3 Controls). This is consistent with previous research highlighting this sequence type as one of community origin (Goswami et al., 2018). Additionally, evidence suggests a strong relationship between $bla_{\text{CTX-M-15}}$ and *E. coli* ST131 (Petty et al., 2014). This proved true for three out of 7 ST131 strains in this study, however, two possessed $bla_{\text{CTX-M-27}}$ and two harboured $bla_{\text{CTX-M-14}}$. These findings demonstrate the presence of ESBL genes and associated sequence types in healthy members of the community that are comparable to those detected in diagnostic and surveillance settings. Furthermore, WGS allowed us to observe that whilst the same ST and resistance gene types persisted in controls, the resistance genes and ST in water users' samples changed between samples taken before the bathing season and samples taken during the bathing season. Whilst the study was not designed to describe this relationship quantitatively, it provides interesting insight into the potential acquisition of AMR bacteria from bathing waters. This study emphasizes the value of ongoing surveillance to increase knowledge on the prevalence and highlight potential concerns of AMR in the general population. Whilst national guidelines exist in Ireland for screening and managing CPE in clinical settings, they fall short of providing a comprehensive understanding of the prevalence and spread of AMR within the wider population.

Many studies report that bathers are at an increased risk of gastrointestinal illness compared to non-bathers (Leonard et al., 2018b; Wade et al., 2003). Nevertheless, heterogeneity in individual study findings is observed, with some studies reporting decreased risks of illness among the exposed population (Arnold et al., 2013; Colford et al., 2007, 2012; Cordero et al., 2012). For example, Arnold et al. (2013), details a lower observation in odds ratio (OR) of gastrointestinal illness in water users 3 days following exposure. Colford et al. (2012) detailed the decreased risk to bathers when partaking in activities in waters classified as good (≤500 cfu/100 mL *E. coli*, ≤200 cfu/100 mL Intestinal Enterococci) when investigating body immersion, head immersion and swallowing water. Similarly, Papastergiou et al. (2012), detailed the decreased risk to bathers in waters classified as excellent. Finally, Cordero et al. (2012), reported a decreased risk of gastrointestinal illness among users of waters classified as excellent, when compared to NWU, with an adjusted odds ratio of 0.88 (95 % CI 0.47–1.63). Whilst the results of these studies cannot be directly compared with the lower observed carriage of AMR bacteria observed in WU from our study, both highlight the variability in the occurrence of illness or carriage of AMR bacteria from exposure to waters meeting or exceeding recommended quality classifications.

An evaluation of water classification methods is required to fully assess potential public health risks of exposure to waterborne organisms beyond those associated with faecal indicator organisms. Research conducted by (Nielsen and Jiang, 2019) has shown that exposure to recreational water, specifically seawater, can significantly alter the users' skin microbiome. The beta-diversity studies conducted by Nielsen and Jiang revealed that whilst the majority of participants had varying microbiomes pre-exposure, they had comparable compositions post-exposure. To the best of the author's knowledge, no such studies have yet been conducted on the effect on the gut microbiome. In the current study, there were no significant differences found in terms of carrying antibiotic-resistant bacteria

(ARB) among individuals engaging in different recreational activities e.g. swimming versus surfing. However, overall, individuals in the WU group were less likely to carry ESBL-PE than controls. This was an unexpected finding, that may relate to differences in other potential exposure between the WU and control group not captured in this study. The Surfer Biome Project in California found that Irish surfers had the highest molecular and metabolomics diversity in comparison to other surfing participants (Kapono, 2018). It is plausible that swimming and other recreation in good quality water, with a low probability of ESBL-PE exposure, may be protective through positive impacts on microbiome diversity. Evidence is emerging to support the relationship between microbiome diversity and reduction in invasion or colonisation probability by enteric pathogens (Panwar et al., 2021; Khan et al., 2021). Furthermore, there is potential that a water quality-dependent dose-response relationship exists, with natural recreational waters having a protective effect in high-quality water, which reverses to increased colonisation risk in lower-quality water for individuals with high exposure risk (Leonard et al., 2018b). A potential limitation of this study is the high proportion of highly educated participants (42 % of participants vs 15 % nationally with a post-graduate qualification), which may indicate a higher than average socio-economic status in the study population (OECD, 2020). However, this limitation is commonly encountered in cross-sectional studies (Enzenbach et al., 2019) and importantly no significant difference in education status was noted between the two study groups. Further research is required to understand the relationship between recreational exposure to bathing waters, the quality of the bathing water and the impact on the human microbiome, and the potential protection this confers against colonisation by AMR Enterobacterales.

5. Conclusion

These results suggest that frequent WU are less likely to carry ESBL-PE compared to controls. Furthermore, this research provides an estimate of the carriage rate of ESBL-PE in healthy individuals in Ireland. The presence of high-risk clones and resistance genes in healthy individuals without recent antimicrobial exposure is a cause for concern. It highlights the extent to which this AMR phenomenon, which was infrequently detected even in the healthcare setting in the late 20th century, is now endemic in the community and the potential for this pattern to be repeated with CPE. This study makes important contributions to the evidence base exploring human interactions with natural environments and their impacts on health. Further research is needed to elucidate the environmental AMR risks and microbiological mechanisms underpinning gut colonisation by AMR bacteria to understand the risk of AMR transmission in natural environments and develop effective mitigation strategies to protect public health.

CRediT authorship contribution statement

Maeve Louise Farrell: Investigation, Formal analysis, Writing – original draft, Visualization. **Alexandra Chueiri:** Investigation, Writing – review & editing. **Louise O'Connor:** Conceptualization, Investigation, Resources, Writing – review & editing. **Sinead Duane:** Conceptualization, Investigation, Writing – review & editing. **Mark Maguire:** Software, Writing – review & editing. **Georgios Miliotis:** Software, Writing – review & editing. **Martin Cormican:** Conceptualization, Writing – review & editing. **Brigid Hooban:** Writing – review & editing. **Anne Leonard:** Writing – review & editing. **William H. Gaze:** Writing – review & editing. **Genevieve Devane:** Writing – review & editing. **Alma Tuohy:** Writing – review & editing. **Liam P. Burke:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Dearbháile Morris:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

Genbank accession code is included in article

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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