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Sewage-associated plastic waste washed up on beaches can act as a reservoir for faecal bacteria, potential human pathogens, and genes for antimicrobial resistance

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ABSTRACT

Sewage-associated plastic wastes, such as wet wipes and cotton bud sticks, commonly wash up on beaches; however, it is unclear whether this represents a public health risk. In this study, sewage-associated plastic waste, and naturally occurring substrates (seaweed and sand), were collected from ten beaches along the Firth of Forth estuary (Scotland, UK) and analysed using selective media for the faecal indicator organisms (FIOs) *E. coli* and intestinal enterococci (IE), and potential human pathogens (*Vibrio* spp.). Minimum inhibitory concentration (MIC) analysis was used to determine antibiotic resistance in selected strains. FIOs and *Vibrio* were more often associated with wet wipes and cotton bud sticks than with seaweed, and there was evidence of resistance to several antibiotics. This work demonstrates that plastics associated with sewage pollution can facilitate the survival and dissemination of FIOs and *Vibrio* and thus, could present an as yet unquantified potential risk to human health at the beach.

1. Introduction

Wastewater treatment plants (WWTPs) are important sources of plastic waste (Gatidou et al., 2019), with an increasing number of reports (and media interest) of sewage-associated plastic waste being discharged directly into the aquatic environment (Mourgkogiannis et al., 2018; Okoffo et al., 2019; Picken and Ellison, 2021). Such items include disposable wet wipes (baby wipes or moist towelettes), cotton bud sticks and sanitary products (e.g., sanitary pads, tampon applicators), which are composed in part, or fully, of plastic polymers, including polyethylene terephthalate (PET), polypropylene (PP) and polyethylene (PE) (Briain et al., 2020). Once in rivers or coastal environments, the organic fraction of sewage effluent is eventually broken down and metabolised by microorganisms. However, sewage-associated plastic waste can wash up and accumulate on beaches, including popular public bathing beaches (Walker et al., 2006; Araújo and Costa, 2007; Storrier et al., 2007; Rapp et al., 2020), with inevitable negative consequences for beach aesthetics, tourism, and local coastal economies (Nelms et al., 2017).

There is currently significant media and public interest in sewage discharge, which corresponds with the increase in the release of untreated sewage being reported globally (e.g., Sydney, Australia; Edinburgh, UK; Los Angeles, USA) (Calderwood, 2021; Picken and Ellison, 2021; Papenfuss, 2022). Such discharge influences environmental and bathing water quality, causes public outrage, and leads to increased reports of adverse health effects (e.g., gastroenteritis, ear, nose and eye infections) (Slack et al., 2021). Although there are strict environmental regulations in place, e.g., in the UK a new Environment Bill was approved in November 2021, these are often breached by water companies who are releasing untreated sewage into the environment in volumes that exceed their permitted discharge limits (Environment Agency, 2021; Environmental Audit Committee, 2022; Stallard, 2022). Blocked sewerage systems reduce the efficiency of WWTPs and can lead to increases in the number of sewage spill events (Briain et al., 2020; Environmental Audit Committee, 2022), with wet wipes responsible for 90% of these blockages (Brown, 2021). With many commercially available brands of wet wipes being labelled as 'flushable' and 14% of people in the UK admitting to incorrectly flushing wet wipes down the toilet (Marine Conservation Society, 2021), it is not surprising that there are increased sewage blockages and subsequent sewage spills. Media

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attention has focused on the accumulation of wet wipes on beaches (Grierson, 2021; Picken, 2021), with the *Great British Beach Clean* reporting an average 25 wet wipes per 100 m on Scottish beaches (Marine Conservation Society, 2021). In addition to signifying recent sewage discharge, sewage-associated plastic waste washing up on beaches can be also an indicator of 'legacy sewage discharge' that has accumulated in the environment from previous discharge events.

Plastics in the environment become rapidly colonised by microbial biofilm (Zettler et al., 2013), and such 'plastisphere' communities are highly variable, diverse and importantly can harbour human pathogens (Galafassi et al., 2021; Wang et al., 2021; Zhang et al., 2021). The protective environment provided by the plastisphere is hypothesised to enhance the survival, persistence, and dissemination of human pathogens in the environment (Keswani et al., 2016). Natural organic materials, such as sand and seaweed, also have the potential to transport microbes, including pathogenic species, in naturally occurring biofilm (Quilliam et al., 2014; Pham et al., 2021). However, there is emerging evidence that suggests that some pathogens can become 'enriched' on plastics compared to natural materials (Pham et al., 2021; Zhao et al., 2021; Metcalf et al., 2022); although conflicting studies have found potential pathogens in higher abundance on other materials (Beloe et al., 2022; Metcalf et al., 2022). Pathogens detected on PET, PP and PE in the environment include *Vibrio* spp. (Jiang et al., 2018; Laverty et al., 2020; Zhang et al., 2021), and the faecal indicator organisms (FIOs) *E. coli* and intestinal enterococci (IE) (Rodrigues et al., 2019; Kelly et al., 2021), which are the key monitoring parameters used for regulating and classifying microbial bathing water quality, e.g., in the EU Bathing Water Directive (Quilliam et al., 2019). Pathogens colonising plastics are often isolated from around sewage discharge sites or other places of anthropogenic activity (Basili et al., 2020; Pazos et al., 2020), and recovery of potential human pathogenic bacteria, such as *Legionella* and *Mycobacterium*, have been reported on plastics inoculated with raw sewage (Wang et al., 2020; Zhao et al., 2021). WWTPs are one of the most important hotspots for human pathogenic bacteria, antimicrobial resistance genes (ARGs) and metal resistance genes (MRGs) (Li et al., 2015; Guo et al., 2017; Martínez-Campos et al., 2021). Sewage-associated plastic waste is likely to be colonised by these bacteria and their associated ARGs and MRGs during their passage through WWTPs (Martínez-Campos et al., 2021); additionally, they can become colonised during the period they remain in the environment. Recreation at bathing waters and on beaches provides opportunities for human exposure to such colonised plastic waste, with the potential for pathogen transfer and subsequent risk to public health.

Due to the high abundance of sewage-associated plastic waste washing up on beaches there is an urgent need to fully understand the risk of this material for facilitating the persistence and transport of human pathogens, particularly in areas with poor wastewater management and high sewage discharges. Therefore, the aim of this study was to quantify the abundance of sewage-associated plastic waste on public beaches in the Firth of Forth estuary (Scotland, UK) and determine whether this waste was colonised by FIOs and *Vibrio* spp., and whether any of these bacteria were expressing ARGs.

2. Materials and methods

2.1. Sampling location

The Firth of Forth is a tidal estuary (tidal range > 5 m) in the east of Scotland (UK), with several nearby urban centres, including the capital city of Edinburgh (population: 518,000). WWTPs are located throughout the catchment, serving approximately 1.6 million people, with 33 plants discharging directly into the estuary, for example, Alloa WWTP, which is just upstream of the study sites, was reported to discharge over 3 million m^3 of untreated sewage between 2016 and 2020 (Picken and Ellison, 2021). Additionally, Seafield WWTP in Leith is Scotland's largest WWTP, processing 300 million L of wastewater every day and serving almost 1 million people (Scottish Water, 2021). There are numerous beaches along the shores of the Forth Estuary, including popular public beaches and EU designated bathing water beaches (Fig. 1).

2.2. Sample collection and processing

Sewage-associated plastic waste (wipes, cotton bud sticks and sanitary products) was collected from ten beaches (Culross, Torryburn, North Queensferry, Aberdour, Burntisland, Portobello, Leith, Cramond, Blackness and Bo'ness) on two consecutive days in December 2021 (Fig. 1; Table S1). At each site a transect (100 m × 10 m) was set out along the hightide strandline, and all sewage-associated plastic waste within the transect collected and placed into sterile ziplocked bags. Due to the high volume of wet wipes at Site 8, a 10 m × 10 m transect was used instead. Naturally occurring substrate, i.e., brown seaweed (*Ascophyllum nodosum*) and sand, were collected at intervals along the transect (0, 50 and 100 m). Estuary water samples (200 mL) at each beach were collected in triplicate in sterile plastic bottles, and temperature and salinity of the water measured in situ at each site. All samples were stored at 4 °C and processed within 48 h. Plastic sewage waste samples were sorted by type (wipes, sanitary products and cotton bud sticks) and weighed. Three representative samples of seaweed, sand, and wipes from each site were placed into a drying oven (Swallow Oven, UK) at 75 °C for 24 h, to determine their dry weights.

Replicate composite samples of wipes (30 g in 100 mL), cotton bud sticks (four sticks in 20 mL) and seaweed (3 g in 20 mL) from each site were pre-enriched in non-selective media Luria-Bertani (LB) broth (Fisher Bio-reagents, UK) or *Vibrio* selective Bile Peptone broth (1% peptone, 0.5% Taurocholic acid, 1% NaCl, pH 9.0 (Faruque et al., 2006)) at 37 °C in a shaking incubator (100 rev min^{-1}; Gallenkamp Orbital incubator) for 18 h. For those sites where less than 30 g of wipes had been collected (i.e., S2–4), 3 g of wipes were placed in 10 mL broth. No cotton bud sticks were present at two sites (S1 and S9) and only two cotton bud sticks were collected from S8, resulting in a composite sample of <4 sticks for this site. Following pre-enrichment, serial dilutions were carried out in phosphate buffered saline (PBS) and dilutions (10^{-4}–10^{-7}) plated onto selective media (membrane lactose glucuronide agar (MLGA) for *E. coli*, thiosulphate citrate bile salts agar (TCBS) for *Vibrio*, and Slantez and Bartley agar (SB) for intestinal enterococci (IE) (Oxoid, UK)). All plates were inverted and incubated; MLGA and TCBS plates were incubated at 37 °C for 24 h and SB plates incubated at 37 °C for 4 h followed by 44 °C for 44 h. The presence or absence of target bacteria on all sewage-associated plastic waste samples was determined by enumerating colony forming units (CFUs).

Samples of estuary water from every site (100 mL, $n = 3$) were directly filtered through 0.45 μm cellulose acetate membrane filters (Sartorius Stedim Biotech., Gottingen, Germany) and aseptically transferred onto the surface of selective media. To remove bacteria associated with the sand samples, 20 mL of sterile PBS was added to 20 g of sand for each replicate sample and shaken for 10 min (100 rev min^{-1}), and vortexed for a further 10 min (1500 rpm). The sand was allowed to settle for a few seconds and then the supernatant was filtered through 0.45 μm cellulose acetate membrane filters and placed on selective media. Following incubation, CFUs were enumerated to quantity the concentration of each target microorganism (100 mL^{-1} water or 100 g^{-1} dry weight sand).

2.3. Multiplex PCR for Vibrio spp. identification

Eight representative colonies (encompassing a range of different colony morphologies and colour) of *Vibrio* spp. from the seaweed (S1, S6), wet wipe (S1, S7), and cotton bud stick (S2, S7) samples were isolated from agar plates and grown overnight in LB broth. DNA extraction was carried out using the QIAamp DNA mini kit (Qiagen, USA), using 250 μL of culture from each sample. Primers from Nhung et al. (2007)

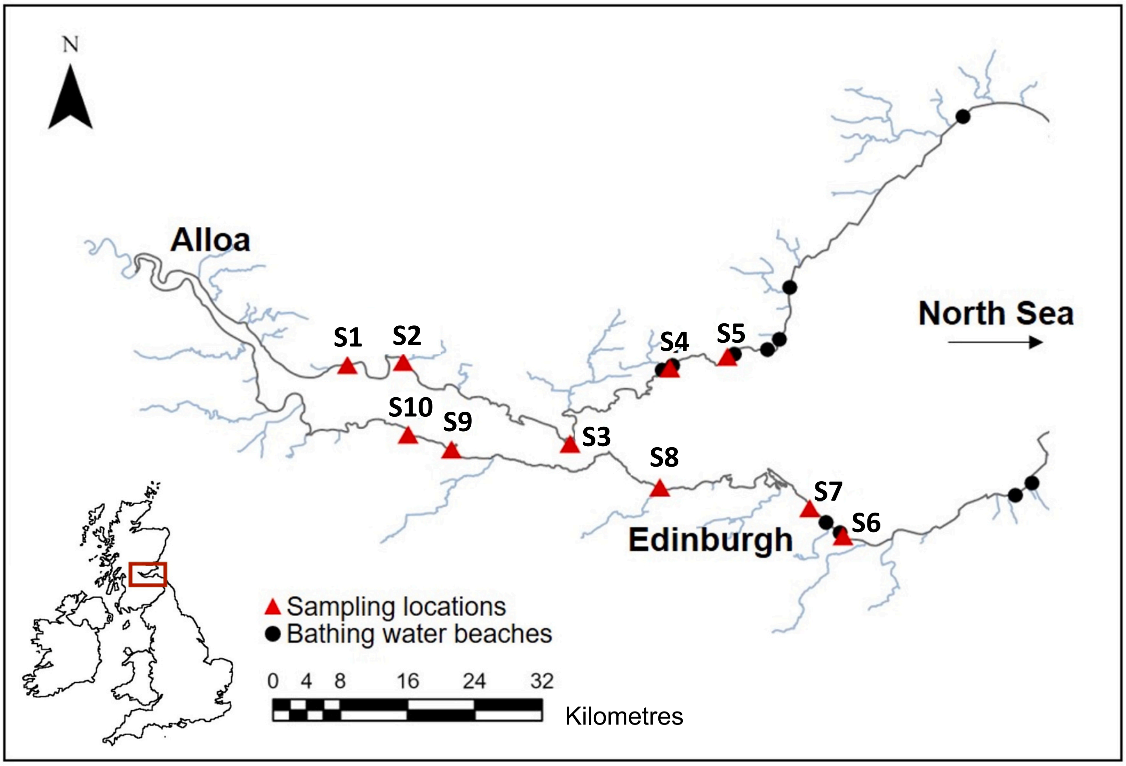

Fig. 1. Sampling sites and EU designated bathing water beaches along the Firth of Forth estuary. Sampling locations (red triangles) are numbered according to Table 1. Bathing water beaches are indicated by black dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

targeting *dnaJ* were used to identify five *Vibrio* species, i.e., *V. alginolyticus*, *V. cholerae*, *V. mimicus*, *V. parahaemolyticus*, and *V. vulnificus*. Amplification reactions consisted of 5 μL Multiplex master mix (New England Biolabs, UK), 3.75 μL of the multiplex primer stock (10 μmol/L) and 5 μL of each DNA sample in a final reaction volume of 25 μL. PCR amplification was carried out in a thermal cycler (Techne TC-412; Keison Products, UK) using the following cycle: 3-minute initial denaturation at 94 °C, followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 7 min. All PCR products were subjected to electrophoresis down a 1.5% agarose gel using GelRed® staining (Biotium, USA) and visualised with UV light. Different amplicon sizes were used to differentiate between distinct *Vibrio* species: *V. alginolyticus* (144 bp), *V. cholerae* (375 bp), *V. mimicus* (177 bp), *V. parahaemolyticus* (96 bp), and *V. vulnificus* (412 bp).

2.4. Minimum inhibitory concentration (MIC)

Representative individual colonies of *E. coli*, IE and *Vibrio* spp. isolated from seaweed, wet wipe, and cotton bud stick samples, were picked off agar plates, grown further overnight in LB broth, and subjected to MIC analysis to determine antibiotic resistance. Resistance or sensitivity to five antibiotics, listed as critically or highly important antimicrobials for human medicine (WHO, 2019), was examined. These included amoxicillin, ampicillin, streptomycin, cephalexin, and tetracycline (at ten concentrations between, 256 mg/L and 0.5 mg/L) (Melford, UK; Duchefa Biochemie, Netherlands). Two-fold serial dilutions of each antibiotic were made in LB broth in 96-well plates. Bacterial cultures were adjusted to an OD_{600nm} of 0.1, and 10 μL added to each well of a 96-well plate. Samples were incubated overnight at 37 °C. The MIC was recorded visually as the lowest concentration of the antimicrobial agent that inhibited bacterial growth (CLSI, 2009; Chen et al., 2021; Paudyal et al., 2021).

2.5. Statistical analysis

Graphs were prepared and statistical analysis conducted using R Studio version 3.3.2 (R Core Team, 2016). Analysis of variance (ANOVA) was used to compare the bacterial concentrations between sites for water and sand. All data were tested for distribution and homogeneity of variances (Shapiro-Wilk and Levene's) before parametric tests were used. Where assumptions were not met, data was square root transformed. Data is reported as mean ± standard error. P values < 0.05 are considered significant.

3. Results

3.1. Quantity of plastic sewage waste

Sewage-associated plastic waste was present on all of the beaches sampled in the Forth Estuary, with higher concentrations found on

Table 1
Quantity of sewage-associated plastic waste collected at each beach.

Site	Wipes		Cotton bud sticks	
	Wet weight (g per 100 m)	Dry weight (g per 100 m)	Weight (g per 100 m)	Number
S1 - Culross	915	385	–	–
S2 - Torryburn	1000	196	2.82	11
S3 - North Queensferry	10.20	5.64	8.37	34
S4 - Aberdour	25.46	15.85	13	52
S5 - Burntisland	13.13	7.45	6.12	24
S6 - Portobello	23.80	10.73	37.89	126
S7 - Leith	162	106	91.25	303
S8 - Crammond	90,003	72,929	1.09	2
S9 - Blackness	3000	1697	–	–
S10 - Bo'ness	938	538	26.76	38

beaches on the south side of the estuary (Table 1). Wet wipes were the most abundant item found, with >70 kg (dry weight) present at Crammond beach within the 100 m transect (extrapolated from a 10 m transect). Beaches further downstream (S3–S7), including the three EU designated bathing waters, had lower quantities of wet wipes, but higher numbers of cotton bud sticks (Table 1). Sanitary products were present on eight of the ten beaches (Table S1). The abundance and composition of sewage-associated plastic waste varied both within and between beaches, but the majority was found accumulated at the hightide strandline (Table 1). Water temperatures ranged from between 3.3 and 6.6 °C and salinity between 0.8 and 3.0% salt. At the point of sampling, over two cold weekdays in December, people (mainly dog walkers and families) were recorded on eight of the beaches (Table S1).

3.2. Bacterial colonisation

At the time of sampling, FIOs and *Vibrio* spp. were detected in the water at all sites, with there being differences in concentration between sites (Fig. 2; ANOVA, Water: $F_{9,60} = 3.734$, $p < 0.001$, Sand: $F_{9,60} = 5.040$, $p < 0.001$). On the day of sampling, the concentration of *E. coli* in the water at all sites was indicative of good water quality under the EU Bathing Water Directive classifications (i.e., ≤500 CFU/100 mL), whilst only eight sites were indicative of good water quality (i.e., ≤200 CFU/100 mL) for IE concentrations. Sand samples had high levels of both FIOs and *Vibrio* spp., with the mean concentrations for each site ranging from 54 to 3815 CFU 100 g^{-1} (dry weight). Wipes had the highest levels of colonisation, with *E. coli* and IE being present on 100% of samples (Fig. 3). Colonisation by *Vibrio* spp. was consistently lower than FIO colonisation across all materials. Colonisation of seaweed by FIOs and *Vibrio* spp. was consistently lower than for wet wipes and cotton bud sticks. Four *Vibrio* species (*V. alginolyticus*, *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*) were detected on the seaweed, wet wipe, and stick samples (Table S2) by PCR, with *V. alginolyticus* present on all materials, and *V. vulnificus* only present on cotton bud sticks. Importantly, this method of species identification did not infer whether these were pathogenic strains of *Vibrio*.

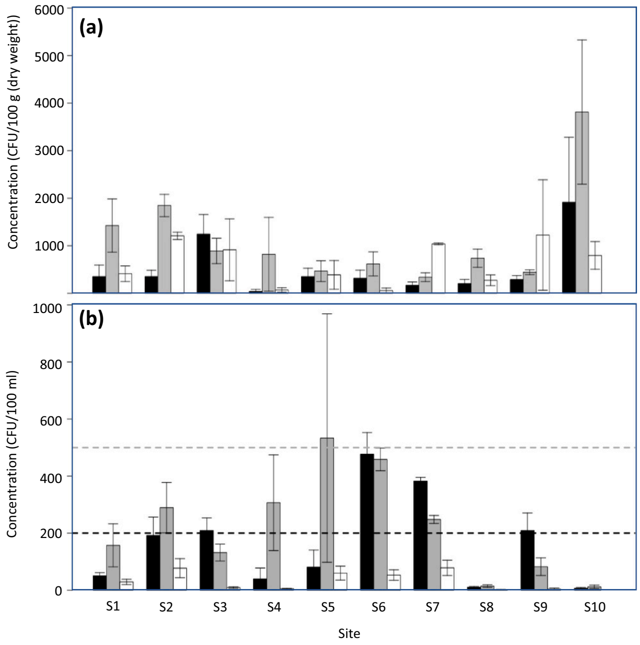

Fig. 2. Concentration of *E. coli* (black bars), Intestinal enterococci (grey bars) and *Vibrio* spp. (white bars) in water (a) and sand (b). The mean was calculated from 3 replicates, ± the standard error. The dashed horizontal lines represent the 'good' water quality classification thresholds for the EU Bathing Water Directive classifications for *E. coli* (black) and IE (grey), based on a 95th percentile evaluation that would be calculated over the entire bathing water season.

3.3. Antibiotic resistance

MIC to five antibiotics were determined for isolates of FIOs and *Vibrio* spp. colonising the surfaces of seaweed, wet wipes, and cotton bud sticks (Fig. 4). The most common forms of resistance were to amoxicillin, ampicillin and cephalexin, with isolates most susceptible to tetracycline. *E. coli* and isolates of *Vibrio* spp. had the highest resistance, although there was little difference in resistance profiles of bacteria isolated from the different materials. However, IE isolated from the wet wipes and cotton bud sticks appeared to be more resistant to the antibiotics tested than those isolated from seaweed. Two thirds of all isolates were resistant to multiple antibiotics, at the concentrations examined (Fig. 4).

4. Discussion

Seven million wet wipes, 2.5 million tampons and 1.5 million sanitary pads are incorrectly flushed down the toilet every day in the UK, resulting in sewage blockages and the release of untreated sewage into the aquatic environment (Environmental Audit Committee, 2022). This results in large quantities of sewage-associated plastic waste accumulating on beaches, and in this study, all beaches sampled were polluted, with wet wipes being the most abundant item found. This waste is not only unsightly but can also present an increased public health risk. FIOs and *Vibrio* spp. were more often associated with sewage-associated plastic waste than seaweed, and there was also evidence of resistance to several antibiotics. This highlights the potential impact this waste could have on human health at beaches through facilitating the survival and dissemination of FIOs and potential human pathogens.

4.1. Sewage-associated plastic waste washed-up on beaches

Most of the anthropogenic litter observed on the sampled beaches was sewage-associated plastic waste, with perhaps surprisingly no evidence of plastic waste associated with the COVID-19 pandemic, e.g., face masks. It has been previously reported that only a small proportion of anthropogenic litter collected on British beaches was sewage waste (5%) (Nelms et al., 2017), which suggests that in recent years the quantity of sewage-associated plastic waste on beaches in the UK has grown, e.g., the quantity of wet wipes washing up on UK beaches is estimated to have increased by 400% in the last decade (Marine Conservation Society, 2019). This is likely due to a rise in the number of sewage spills, which have increased by 40% in Scotland in the last five years (Slack et al., 2021; Environmental Audit Committee, 2022). Thirty-three WWTPs directly release treated wastewater effluent into the Forth Estuary; this includes Alloa WWTP, which according to recent media reports is one of the top 10 locations in Scotland for the volume of sewage discharge (Picken and Ellison, 2021). Once discharged, sewage waste travels downstream, accumulates in the estuary, and is washed-up on the beaches due to local wind and tidal patterns, which was evident by the abundance and composition of collected sewage-associated plastic waste varying with location, both within and between beaches. This is likely to be a result of coastal currents and bidirectional tidal flows within the Forth Estuary, which control the transport and accumulation of plastic waste (Lam et al., 2020; Pinheiro et al., 2021); the estuary has a large tidal range of up to 5.8 m and a tidal flux up to 30,000 m^3/s (Elliott and Neill, 2007). Estuaries often entrap and accumulate plastic waste, where it either remains suspended or sinks to the estuary bed where it can become incorporated into sediments (Lam et al., 2020; Pinheiro et al., 2021; Schernewski et al., 2021). Plastics can remain 'trapped' within the estuary over a range of timescales before being washed up onto beaches; therefore, the accumulation of sewage-associated plastic waste on the beaches in this study could be an indication of 'legacy' sewage discharge over long timescales. The beach

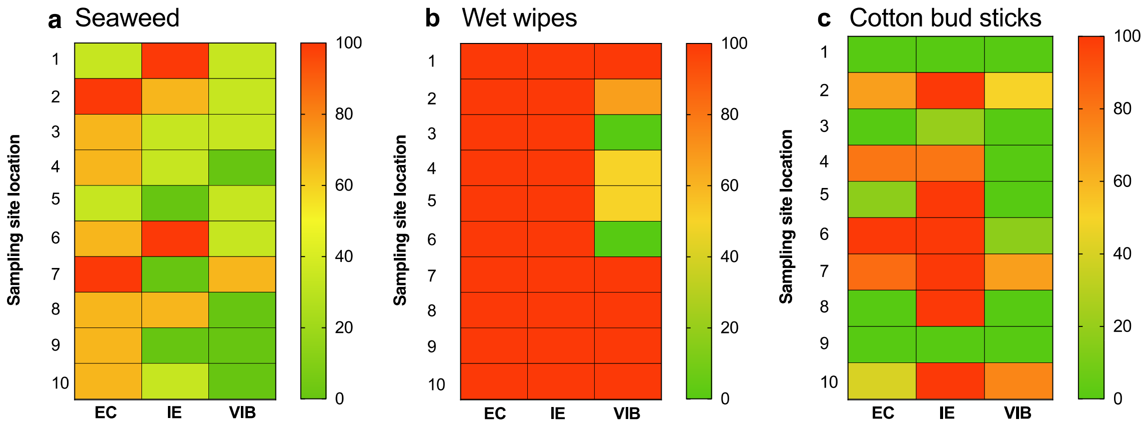

Fig. 3. FIOs and *Vibrio* spp. recovered from seaweed, wet wipes and cotton bud sticks in the Forth estuary. Using selective media, FIO and *Vibrio* spp. were identified on (a) seaweed, (b) wet wipes and (c) cotton bud sticks from the ten sampled sites along the Firth of Forth. The colour scale indicates the percentage of material that was colonised by each species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

location and geomorphology, as well as the western prevailing winds and weather conditions will all have influenced where the waste was deposited and the rate of its accumulation on the shores of the estuary (Ghaffari et al., 2019; Pinheiro et al., 2019; Jong et al., 2022; Nelms et al., 2017).

4.2. *Sewage-associated plastic waste on beaches are colonised by FIOs and Vibrio*

Sewage-associated plastic waste on the beaches of the Firth of Forth estuary were colonised by both FIOs and species of *Vibrio*. Three of the beaches sampled are EU designated bathing water beaches (Aberdour, Burntisland and Portobello), where human exposure to sewage waste will be higher during the bathing water season, or for beach users outside of the bathing water season, such as wild swimmers, or during recreational water sports. FIOs are likely to have colonised sewage-associated plastic waste as it passed through WWTPs (Guo et al., 2017; Martínez-Campos et al., 2021), but opportunities for additional colonisation could occur as plastics move through faecally-contaminated water (contaminated from either sewage discharge or agricultural run-off), or through direct contact on beaches (e.g., by dog or bird faeces). It is unclear how long different bacterial species can survive and persist within the plastisphere of sewage-associated plastic waste, but survival could be facilitated by the protective environment provided by the biofilm (Keswani et al., 2016).

Both FIOs and *Vibrio* spp. were more often associated with sewage-associated plastic waste than with seaweed, and potentially pathogenic species of *Vibrio* (e.g., *V. parahaemolyticus*) were isolated from the plastisphere on most of the beaches sampled. Although, multiplex PCR can differentiate between species of *Vibrio*, it is unable to determine virulence (and therefore risk) and testing for virulence genes would be required to determine actual pathogenesis (Wright et al., 2020). Bacteria are ubiquitous on beaches, colonising both sand and seaweed, which can provide a more readily available source of nutrients compared to plastics (Quilliam et al., 2014; Song et al., 2020), and FIOs and potential pathogens can become enriched on the surfaces of both plastics and natural materials, such as leaves, feathers and sand (Metcalf et al., 2022). However, the distinct properties of plastics (e.g., buoyancy and durability) could allow bacteria colonising these surfaces to persist for longer and be more widely transported within the environment.

4.3. *Antibiotic resistance*

Bacteria isolated from plastic samples in this study showed resistance to several antibiotics. Horizontal gene transfer and contaminants on plastic surfaces are thought to increase the development of antimicrobial resistance (Teuten et al., 2007; Arias-Andres et al., 2018; Vos, 2020). Reports have shown that plastisphere pathogens can be most resistant to amoxicillin and ampicillin and least resistant to tetracycline (Moore et al., 2020; Liu et al., 2021), and here we have identified similar patterns of resistance to these antibiotics. Such resistance causes concern for human health; ampicillin is one of the most widely used antibiotics for the treatment of IE infections (Gavalda et al., 2007). Antimicrobial resistance is now considered an important global threat to human public health, and it is predicted that antimicrobial resistance will be a major cause of mortality by 2050 (Dadgostar, 2019). With sewage-associated plastic waste acting as a reservoir of FIOs and ARGs, the opportunity for increased environmental dissemination and subsequent pathways for human exposure will be increased at beaches and in coastal waters.

5. Conclusion

The release of untreated sewage into aquatic environments is commonly reported in the media, by NGOs and in the literature (e.g., McCoy et al., 2020; Slack et al., 2021). Improved infrastructure and associated funding provision, together with increased monitoring and management, is required to prevent (or at least reduce) the release of untreated sewage into the aquatic environment. Sewage blockages are partly responsible for an increase in the number of sewage spills; they are primarily caused by plastic waste, including wet wipes, and annually cost water companies £100 million to clear (Environmental Audit Committee, 2022). Although businesses and private households are encouraged not to dispose of items that block sewers, millions of items are incorrectly flushed down toilets every day in the UK (Environmental Audit Committee, 2022). The demand for wet wipes has increased as a result of the COVID-19 pandemic (Hu et al., 2022), therefore, now more than ever, there is an urgent need for greater investment in public awareness programmes to eliminate wet wipes entering sewerage systems. FIOs and species of *Vibrio* were more often associated with wet wipes on the beaches of the Forth Estuary than with seaweed, and there was evidence of resistance to several antibiotics. This work has demonstrated that plastics associated with sewage pollution can facilitate the survival and dissemination of harmful microorganisms and as such could present an increased potential risk to human health at the beach.

CRediT authorship contribution statement

Rebecca Metcalf: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Hannah L. White:** Conceptualization, Methodology, Investigation,

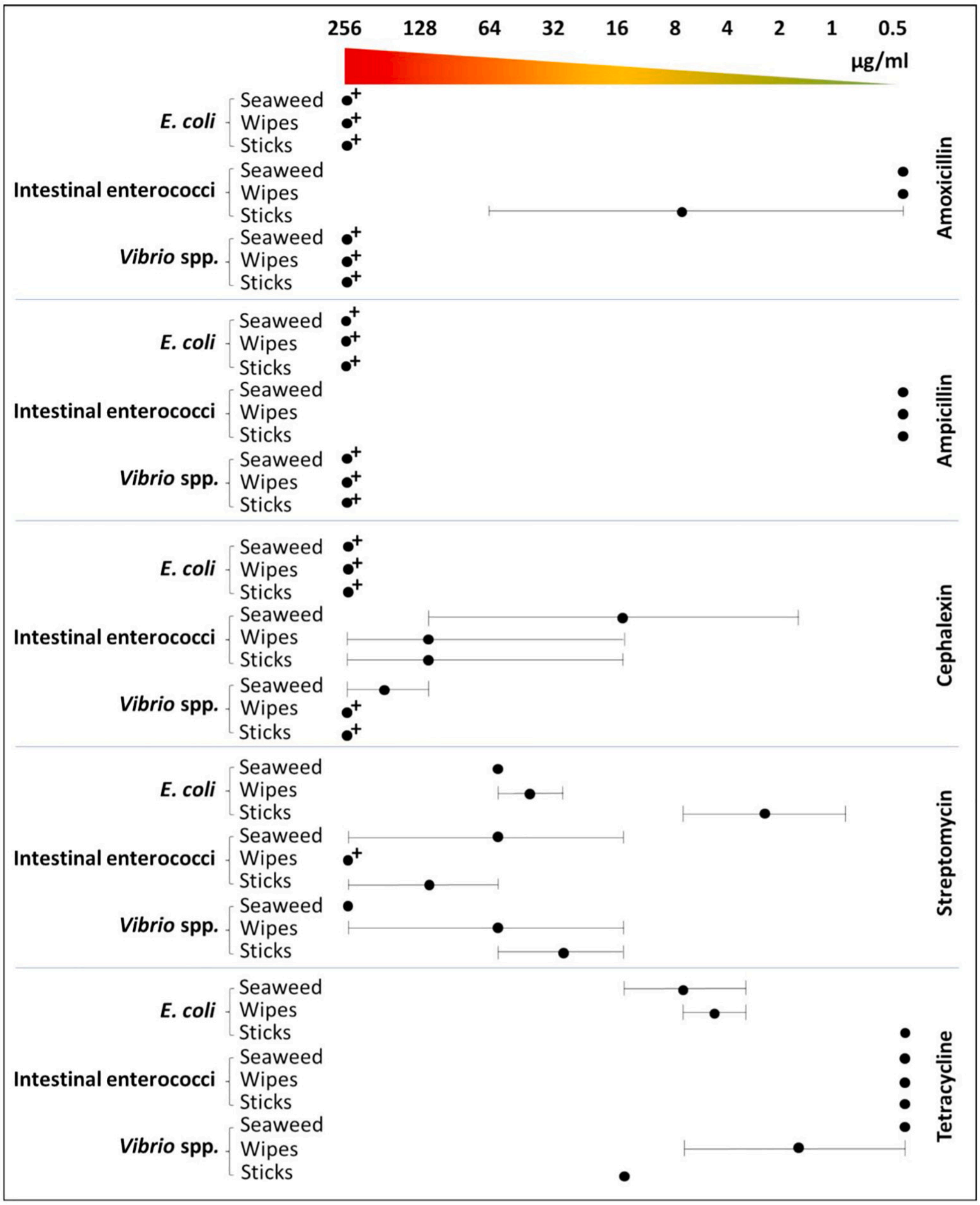

Fig. 4. Minimum Inhibitory Concentration (MIC) of *E. coli*, Intestinal enterococci and *Vibrio* spp. recovered from beaches in the Forth Estuary. Strains able to grow at the highest concentration (+) indicates that their MIC would probably be higher than this. There were three replicates per treatment, error bars indicate the range.

Writing – review & editing. **Vanessa Moresco:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Michael J. Ormsby:** Conceptualization, Methodology, Investigation, Writing – review & editing. **David M. Oliver:** Conceptualization, Methodology, Writing – review & editing. **Richard S. Quilliam:** Conceptualization, Methodology, 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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Data availability

Data is available at University of Stirling DataSTORRE (http://hdl.handle.net/11667/196).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.113766.

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