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Exposure to and colonisation by antibiotic-resistant *E. coli* in UK coastal water users: Environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey)

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ABSTRACT

Background: Antibiotic-resistant bacteria (ARB) present a global public health problem. With numbers of community-acquired resistant infections increasing, understanding the mechanisms by which people are exposed to and colonised by ARB can help inform effective strategies to prevent their spread. The role natural environments play in this is poorly understood. This is the first study to combine surveillance of ARB in bathing waters, human exposure estimates and association between exposure and colonisation by ARB in water users.
Methods: 97 bathing water samples from England and Wales were analysed for the proportion of *E. coli* harbouring $bla_{CTX\text{-}M}$. These data were used to estimate the likelihood of water users ingesting $bla_{CTX\text{-}M}$-bearing *E. coli*. Having identified surfers as being at risk of exposure to ARB, a cross-sectional study was conducted. Regular surfers and non-surfers were recruited to assess whether there is an association between surfing and gut colonisation by $bla_{CTX\text{-}M}$-bearing *E. coli*.
Results: 11 of 97 bathing waters sampled were found to contain $bla_{CTX\text{-}M}$-bearing *E. coli*. While the percentage of $bla_{CTX\text{-}M}$-bearing *E. coli* in bathing waters was low (0.07%), water users are at risk of ingesting these ARB. It is estimated that over 2.5 million water sports sessions occurred in 2015 resulting in the ingestion of at least one $bla_{CTX\text{-}M}$-bearing *E. coli*. In the epidemiological survey, 9/143 (6.3%) surfers were colonised by $bla_{CTX\text{-}M}$-bearing *E. coli*, as compared to 2/130 (1.5%) of non-surfers (risk ratio = 4.09, 95% CI 1.02 to 16.4, $p = 0.046$).
Conclusions: Surfers are at risk of exposure to and colonisation by clinically important antibiotic-resistant *E. coli* in coastal waters. Further research must be done on the role natural environments play in the transmission of ARB.

1. Introduction

There is little doubt that the extensive anthropogenic use of antibiotics and antimicrobials has accelerated the emergence of antibiotic resistance among bacteria (Davies and Davies, 2010; Hawkey and Jones, 2009). Resistance can arise by mutation or be acquired by horizontal transfer of resistance genes on mobile genetic elements (MGEs) such as plasmids, from one bacterium to other members of a bacterial community. As many essential antibacterial agents fail to treat diseases caused by antibiotic-resistant bacteria (ARB), and with only a limited number of antibiotics to which they may still be susceptible remaining, resistance has been described as “one of the greatest health threats faced today” (Davies et al., 2011). If current trends continue, rates of morbidity and mortality from infections caused by ARB will increase (de Kraker et al., 2011; European Centre for Disease Prevention and Control and European Medicines Agency, 2009). In the recent ‘O'Neill Review on Antimicrobial Resistance’ it was estimated that by the year 2050, drug-resistant infections could cause 10 million human deaths globally every year (Review on antimicrobial resistance, 2014).

One group of ARB that is of special concern in human medicine are extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, which have been identified by the World Health Organization as being a

Abbreviations: MGE, mobile genetic element; ARB, antibiotic resistant bacteria; ESBL, extended-spectrum beta-lactamase; $bla_{CTX\text{-}M}$, beta lactamase cefotaximase-Munich; 3GCs, third-generation cephalosporins; PCR, polymerase chain reaction; 95% CI, 95% confidence interval; UTI, urinary tract infection; UK, United Kingdom

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"critical priority" for the research and development of new antibiotics (World Health Organization, 2017). Bacteria producing ESBL enzymes are able to grow and survive in the presence of various β-lactam antibiotics, which include a wide range of clinically useful medicines such as penicillins and cephalosporins (Nordmann et al., 2012). Plasmids carrying ESBLs, like bla_{CTX-M}, can be mobilised between bacteria, often conferring resistance to multiple antibiotics, for example fluoroquinolones, aminoglycosides, and tetracyclines (Johnson et al., 2010; Livermore and Hawkey, 2005; Nordmann et al., 2012). Although there are a number of different plasmid-borne ESBLs (including bla_{TEM} and bla_{SHV}), bla_{CTX-M} genes represent nearly 80% of ESBLs in clinical isolates, with $bla_{CTX-M-15}$ being the most common genotype found worldwide (Amos et al., 2014). Worryingly, their prevalence is increasing outside healthcare settings (Amos et al., 2014; Hawkey and Jones, 2009). The rapid emergence and spread of ESBLs (particularly the CTX-Ms) poses a significant public health threat, as infections caused by ESBL-producing bacteria are unresponsive to multiple antibiotics, including essential frontline drugs such as third-generation cephalosporins (3GCs) (Collignon et al., 2009). Carbapenems are one of the few classes of antibiotics recommended for treating infections caused by ESBL-producing bacteria, and while resistance to these last-resort antibiotics remains rare in the UK (European Centre for Disease Prevention and Control, 2014), the numbers of infections caused by carbapenemase-producing *Enterobacteriaceae* has risen since 2010 (European Centre for Disease Prevention and Control, 2016).

Escherichia coli (a group of bacteria within the *Enterobacteriaceae* family) have a complex phylogenetic substructure (Clermont et al., 2013). Many are harmless commensals inhabiting the intestines of healthy animals, including humans (Nicolas-Chanoine et al., 2014). However, some types cause intestinal and extra-intestinal infections. The phylogenetic groups B2 and D contain numerous extra-intestinal pathogenic *E. coli* which can cause serious infections. For example, *E. coli* is the predominant pathogen responsible for community-acquired urinary tract infections (UTIs), but can also cause meningitis and is the commonest cause of bloodstream infections in humans (European Centre for Disease Prevention and Control, 2014). Recently a highly virulent and resistant uropathogenic strain of *E. coli*, O25b-ST131, has emerged and is spreading worldwide (Clermont et al., 2009).

The processes by which people acquire ARB have been the subject of much research. Human exposure to ARB via contaminated food and water occurs in many contexts, such as healthcare settings, at home, and during international travel (Coleman et al., 2012; Kennedy and Collignon, 2010; Paltansing et al., 2013; Valverde et al., 2008). Natural environments have been recognised as a potential but understudied setting in which members of the public come into contact with ARB and where transmission of ARB to humans may occur (Ashbolt et al., 2013; Manaia, 2017). Manure applied as fertiliser to crops, and wastewater discharged into waterways introduce large numbers of bacteria carrying diverse MGEs to coastal waters, along with compounds that select for resistant microorganisms (Amos et al., 2014). Furthermore, coastal locations in Great Britain receive millions of visits annually, and bathing waters may be an important setting in which people come into direct contact with ARB, particularly when participating in water sports. A recent systematic review of the risks of illness caused by sea bathing in high-income countries demonstrated a significant increase in the risk of experiencing symptoms of gastrointestinal infection among bathers compared to non-bathers (Leonard et al., 2018). There is also evidence that ingestion of water containing antibiotic-resistant *E. coli* is associated with gut colonisation by these bacteria (Coleman et al., 2012), and that swimming is a risk factor for urinary tract infections caused by ESBL-producing bacteria (Soraas et al., 2013). Therefore, bathers swallowing ARB in coastal waters could become colonised by ARB and contribute to the prevalence of ARB in the community.

In the first of its kind, this study aimed to quantify the prevalence of bla_{CTX-M}-bearing *E. coli* in bathing-associated waters, to estimate the exposure risk that water users face, and to determine if there is an association between surfing in coastal waters and gut colonisation by antibiotic-resistant *E. coli*.

2. Methods

This study is described in three sections: 1) environmental monitoring (Section 2.1), in which bathing waters were analysed for the proportion of *E. coli* harbouring $bla_{CTX-M,}$ 2) estimating the risk of exposure to bla_{CTX-M}-bearing *E. coli* among coastal water users (Section 2.2), and 3) a cross-sectional survey to estimate the proportion of surfers and non-surfers colonised by these resistant *E. coli* (Section 2.3). The methods in each section are described briefly below, but further details can be found in the study protocol (Appendix).

2.1. Environmental monitoring

2.1.1. Bathing water sampling

97 bathing water samples were collected by the Environment Agency from England and Wales between 3rd July and 27th September 2012 as part of their routine water quality monitoring (Porter, 2012). Water samples were transported on ice to the laboratory for analysis.

2.1.2. Quantifying the proportion of phenotypic resistance to third-generation cephalosporins among E. coli

Culture-based methods were used to isolate and quantify the proportion of *E. coli* in the water samples that were phenotypically resistant to the minimum inhibitory concentrations of 3GC antibiotics cefotaxime and ceftazidime (EUCAST, 2014), as previously described (Leonard et al., 2015).

2.1.3. Quantifying the proportion of bla_{CTX-M} carriage among E. coli, and characterising CTX-M gene diversity

In 2013, colonies resistant to 3GCs were picked and colony PCRs were performed using universal primer pairs to detect the presence of the bla_{CTX-M} gene family. Then genotype-group specific primer pairs for groups 1, 2, 8, 9, and 25 genes were used to amplify the bla_{CTX-M} genes before sequencing (GATC Biotech). DNA sequences were aligned with known sequence variants (from GenBank®) using MEGA7 to identify the genotype (Kumar et al., 2015; National Center for Biotechnology Information, 2016).

2.1.4. Phylotyping E. coli colonies and detecting the pathogenic O25b-ST131 clone

E. coli phylogroup typing was performed on bla_{CTX-M}-bearing colonies (Clermont et al., 2013), and *E. coli* O25b-ST131 clones were detected by PCR (Clermont et al., 2009; Johnson et al., 2009).

2.2. Estimating the risk of exposure to bla_{CTX-M} -bearing E. coli among coastal water users

To demonstrate that water users are at risk of exposure to ARB in coastal waters, methods similar to those described previously were used to estimate the average number of bla_{CTX-M}-bearing *E. coli* that various water users ingested in coastal waters in 2015 (Leonard et al., 2015). Briefly, weekly *E. coli* density data were obtained for all 415 English and 104 Welsh coastal waters in the 2015 bathing season (mid-May to the end of September) from the Environment Agency and Natural Resources Wales. Data for 2015 were selected as the time period during which bathers participating in the epidemiological survey (Section 2.3) would have been most recently exposed to resistant *E. coli*. Data from Section 2.1.3 on the proportion of *E. coli* harbouring bla_{CTX-M} were used to estimate the mean number of bla_{CTX-M}-bearing *E. coli* present in coastal bathing waters. Estimates of the volume of water that water users ingest were obtained from a review of the literature and used to calculate the average number of bla_{CTX-M}-bearing *E. coli* the typical

water user swallows during a session of their chosen activity. Using reported numbers of beach activities in England and Wales, the likely number of exposure events (sessions of water sport that resulted in the ingestion of at least one bla_{CTX-M}-bearing *E. coli*) that occurred in 2015 was also calculated.

2.3. The Beach Bum Survey: a cross-sectional survey to investigate the link between surfing and gut colonisation by antibiotic resistant bacteria

2.3.1. The epidemiological survey

This cross-sectional study aimed to investigate frequent surfers, body boarders and body surfers (hereafter referred to as 'surfers') as the group exposed to coastal waters, alongside people with very little exposure to seawater as the 'control' group. Planning began in 2014. To detect a difference of 15 percentage points (25% versus 10%, respectively) in the prevalence of gut colonisation by bla_{CTX-M}–bearing *E. coli* with 90% power at the 5% (2-tailed) level of significance, it was estimated that 150 surfers and 150 matched controls would be needed. The prevalence of gut colonisation in the wider community (10%) was obtained from Wickramasinghe et al. (2012). Participants were recruited via Surfers Against Sewage (https://www.sas.org.uk/), an environmental charity located in the Southwest UK, between April 2015 and October 2015. The primary method of participant recruitment was conducted using Surfers Against Sewage's network of regional representatives (Reps) to recruit 150 surfers. The Reps were asked to communicate the existence and purpose of the Beach Bum Survey to surfers in their area, and to recruit interested people to the study. Following extensive media coverage of the survey in June 2015, many volunteers contacted Surfers Against Sewage (SAS) to express interest in taking part. An employee at SAS sent interested individuals further information on the study and screened them for eligibility. Once confirmed as eligible, volunteers were sent kits along with the information sheet, a consent form, a rectal swab (Medical wire, Fecal Transwab Cary Blair Media), and a short questionnaire. Participating surfers were also asked to invite a non-bathing friend who was of a similar age, same sex, and lived in the same region of the UK to take part in the study as their matched controls. Eight months into data collection, the desired sample size had not been achieved, and SAS introduced a reward scheme for their Reps whereby they were rewarded with merchandise for recruiting people to the study. Further recruitment efforts were made at the Eden Project, as well as at the Newquay Marine Group, and Plymouth Marine Laboratory. Participants were asked to collect their own rectal swab and to submit it to our laboratory in a pre-paid envelope for analysis along with their completed anonymous questionnaire (Table S3). The questionnaire collected some demographic information, data on the participant's exposure to coastal waters and various risk factors thought to be associated with gut colonisation by ARB (such as diet, travel outside the UK, and domestic and occupational exposures to human or animal faeces).

2.3.2. Estimating the proportion of surfers and non-surfers colonised by antibiotic-resistant bacteria

Enteric bacteria collected on the swabs were tested for their resistance to the clinical breakpoint concentrations of the 3GC antibiotic, cefotaxime, and a carbapenem antibiotic, meropenem (EUCAST, 2014). Culture-based methods were used to isolate cefotaxime-resistant bacteria and meropenem-resistant bacteria from each subject, and each participant was recorded as being colonised by ARB if *E. coli* grew in the presence of either antibiotic. Cefotaxime-resistant strains were screened for the bla_{CTX-M} gene, and meropenem-resistant isolates were screened for carbapenemase genes commonly found in *Enterobacteriaceae* (bla_{NDM}, bla_{KPC}, bla_{VIM} or bla_{OXA-48}) using real-time PCR. As in Section 2.1.4, bla_{CTX-M} gene diversity was characterised and *E. coli* phylogroups identified. *E. coli* O25b-ST131 clones were detected by PCR.

2.3.3. Data analysis

The exposure of interest was a dichotomous variable: recreational exposure to coastal waters or not. This was determined by whether or not the respondents self-identified as a frequent surfer (surfs at least three times per month) or as a control subject (very little exposure to coastal waters). The risk ratios for the presence of cefotaxime-resistant *E. coli* and bla_{CTX-M}-bearing-*E. coli* were estimated, and 95% test-based confidence intervals (CI) were reported.

Potential risk factors for gut colonisation by ARB (for example diet, domestic and occupational exposures to human or animal faeces), were assessed for their association with both the exposure variable and outcome variables. Approval for the study was obtained from the University of Exeter Medical School Research Ethics Committee (reference number 15/02/067).

3. Results

3.1. Results of the environmental monitoring

Of 97 sites sampled, 15 (15%) were found to contain 3GC-resistant *E. coli* and 11 (11%) had bla_{CTX-M}-bearing *E. coli*. All but one region (Yorkshire and the Humber) were found to have 3GC-resistant *E. coli* present, and the prevalence of resistant *E. coli* was similar across regions (Fig. 1). Similar levels of resistance were also observed over the sampling period (July to September) (Fig. S1). Across England and Wales, 114,917 *E. coli* were isolated on non-selective agar: 140 (0.12%) *E. coli* were 3GC-resistant, and 83 (0.07%) *E. coli* were found to harbour bla_{CTX-M} genes (Table S4).

Fifty eight of the 83 bla_{CTX-M}-bearing *E. coli* were further characterised to identify the bla_{CTX-M} genotype and host *E. coli* phylogroup. However, 25 bla_{CTX-M}-bearing *E. coli* isolates were lost during sub-culturing and storage and it was not possible to amplify the entire bla_{CTX-M} gene for genotype identification for six of the isolates. The majority (86.2%) of bla_{CTX-M} genes that were characterised were identified as being CTX-M-15, although CTX-M-1 and CTX-M-27 were also found (Table 1). Four further genotypes were present in *Enterobacteriaceae* isolated from two reference wastewater samples (Table S4) but were not present in bathing water-associated *E. coli*. Isolates belonging to phylogroup B2 were the most prevalent phylogroup at 29.3% of the bla_{CTX-M}-bearing *E. coli* isolates from bathing water-associated samples. Of the 17 colonies identified as belonging to phylogroup B2, 11 (64.7%) were *E. coli* O25b-ST131 (Table 1).

3.2. The risk of exposure to bla_{CTX-M}-bearing E. coli among coastal water users

The results of the environmental assessment (Section 3.1) indicate that on average, 0.07% of *E. coli* in coastal waters of England and Wales harbour bla_{CTX-M} genes. The average number of bla_{CTX-M}-bearing *E. coli* that water users ingest per session is presented in Table 2. Applying these estimates to population-level data, 2,536,932 water sports sessions occurred in England and Wales in 2015 that resulted in people ingesting at least one bla_{CTX-M}-bearing *E. coli* (Table S5). These results indicate that there is a risk of water users swallowing bla_{CTX-M}-bearing *E. coli*, and that surfers are at a particularly high risk of being exposed.

3.3. Results of the Beach Bum Survey: a cross-sectional survey to investigate the link between surfing and gut colonisation by antibiotic resistant bacteria

Of the 294 volunteers who submitted data, 273 were suitable for inclusion in the study: 143 from surfers and 130 from controls (Fig. 2).

Of the 143 surfers, 13 (9.1%) were faecal carriers of *E. coli* that were phenotypically resistant to cefotaxime, as compared to four of the 130 controls (3.1%), giving a risk ratio of 2.95 (95% CI 1.05 to 8.32, $p = 0.040$) (Table 3), and nine (6.3%) surfers were found to be carriers of bla_{CTX-M}-bearing *E. coli*, as compared to two (1.5%) controls (risk

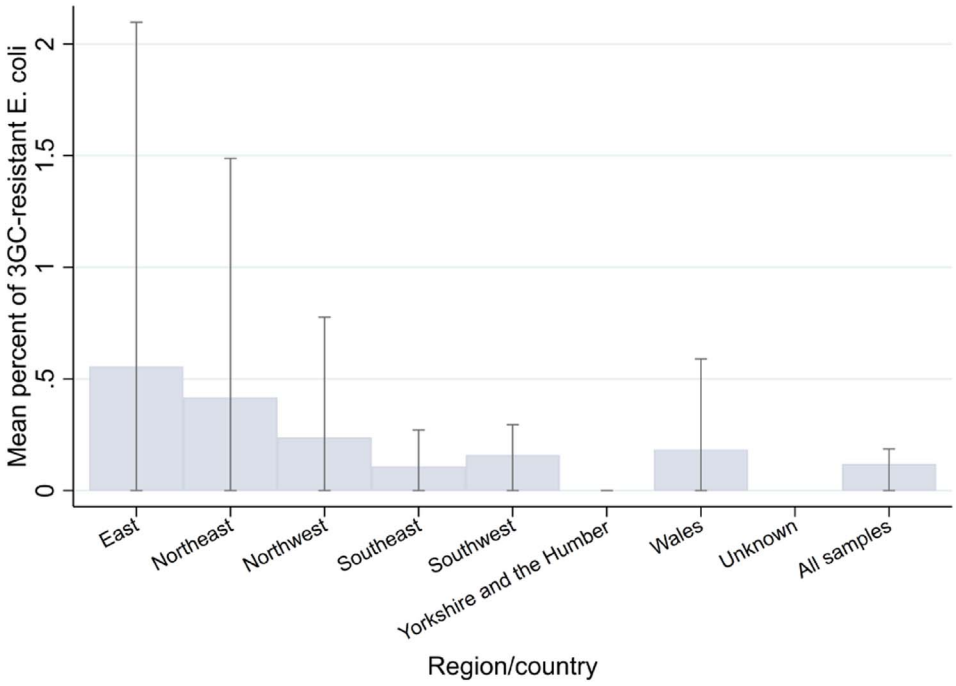

Fig. 1. Bar chart of the mean percent of *E. coli* that were 3GC-resistant in sites sampled within different regions of England and Wales. Error bars indicate 95% confidence limits.

Table 1
Phylogenetic groups of bla_{CTX-M}-bearing *E. coli* isolated from bathing water-associated samples and bla_{CTX-M} gene diversity. Numbers in parentheses indicate the number of bla_{CTX-M}-bearing *E. coli* O25b-ST131 colonies identified. It was not possible to characterise 25 of the 83 bla_{CTX-M}-bearing *E. coli*.

E. coli phylogroups	Number of bla_{CTX-M}-bearing *E. coli* isolated from bathing water-associated samples			
	CTX-M-1	CTX-M-15	CTX-M-27	CTX-M (total)
A	1	11	0	12
B1	1	0	0	1
B2 (*E. coli* O25b-ST131)	0	13 (11)	4	17 (11)
C	0	4	0	4
D	0	5	0	5
E	0	0	0	0
F	2	11	0	13
Unknown	0	6	0	6
Total	4	50	4	58

Table 2
The mean number of bla_{CTX-M}-bearing *E. coli* that various water users ingest per session in bathing waters of different quality. *Water quality categories were based on the *E. coli* density thresholds reported by the revised European Bathing Water Directive (2006/7/EC). *E. coli* units are reported as colony forming units (CFU) per 100 ml.

Activity	Mean number of bla_{CTX-M}-bearing *E. coli* ingested per session of each water sport		
	Guideline* (0–250 *E. coli* CFU/100 ml)	Mandatory* (251–500 *E. coli* CFU/100 ml)	Fail* (> 500 *E. coli* CFU/100 ml)
Swimming (adults)	0.003	0.040	0.260
Swimming (non-adults)	0.007	0.092	0.600
Surfing	0.032	0.424	2.769
Diving	0.002	0.025	0.161
Boating	0.001	0.010	0.060
Canoeing	0.001	0.010	0.063
Fishing	0.001	0.009	0.058
Kayaking	0.001	0.009	0.057
Rowing	0.001	0.009	0.062
Wading	0.001	0.009	0.060

ratio = 4.09, 95% CI 1.02 to 16.4, $p = 0.046$) (Table 3).

Risk factors collected by the questionnaire were assessed for their association with being a surfer (Table 4). There were no major differences in the distribution of risk factors between surfers and controls, with the exception of sex, age, exposure to coastal environments, and doing water sports in non-coastal environments: surfers tended to be males and younger, and tended to have been in the sea recently, and more frequently. They also tend to do non-water sports recreational activities (such as walking, fishing, sunbathing, and rock pooling) at beaches compared to the controls. However, none of the investigated risk factors were found to also be associated with either outcome (carriage of cefotaxime-resistant *E. coli* or bla_{CTX-M}-bearing *E. coli*). For this reason, and because too few participants had the outcome of interest, risk metrics were not adjusted for confounders.

Again, most (90.1%) CTX-M-producing *E. coli* isolated from survey participants carried $bla_{CTX-M-15}$ (Table 5), although one subject was colonised by CTX-M-57-producing *E. coli*. As with the bathing water, most bla_{CTX-M}-bearing *E. coli* isolated from the volunteers belonged to phylogenetic group B2, and all of these were *E. coli* O25b-ST131.

Meropenem resistance was rare among the bacteria isolated from human subjects. No meropenem-resistant *E. coli* were isolated, however one surfer (0.7%) and one control (0.8%) were colonised by meropenem-resistant *Enterobacter cloacae* (Table S6). No association was identified between surfing and gut colonisation by meropenem-resistant bacteria (risk ratio = 0.91, 95% CI 0.06 to 14.4, $p = 0.946$). None of these isolates contained the carbapenemase genes commonly found in *Enterobacteriaceae* (bla_{NDM}, bla_{KPC}, bla_{VIM} or bla_{OXA-48}).

4. Discussion

Escherichia coli (including those belonging to the virulent ST131 clonal lineage) harbouring bla_{CTX-M} are present in coastal waters of England and Wales. While the percentage of *E. coli* in bathing waters harbouring bla_{CTX-M} is low (0.07%), levels are high enough to pose an exposure risk to water users, with over 2.5 million exposure events estimated to have occurred in 2015. One group of water users, surfers, were found to be at a particularly high risk of exposure to bla_{CTX-M}-

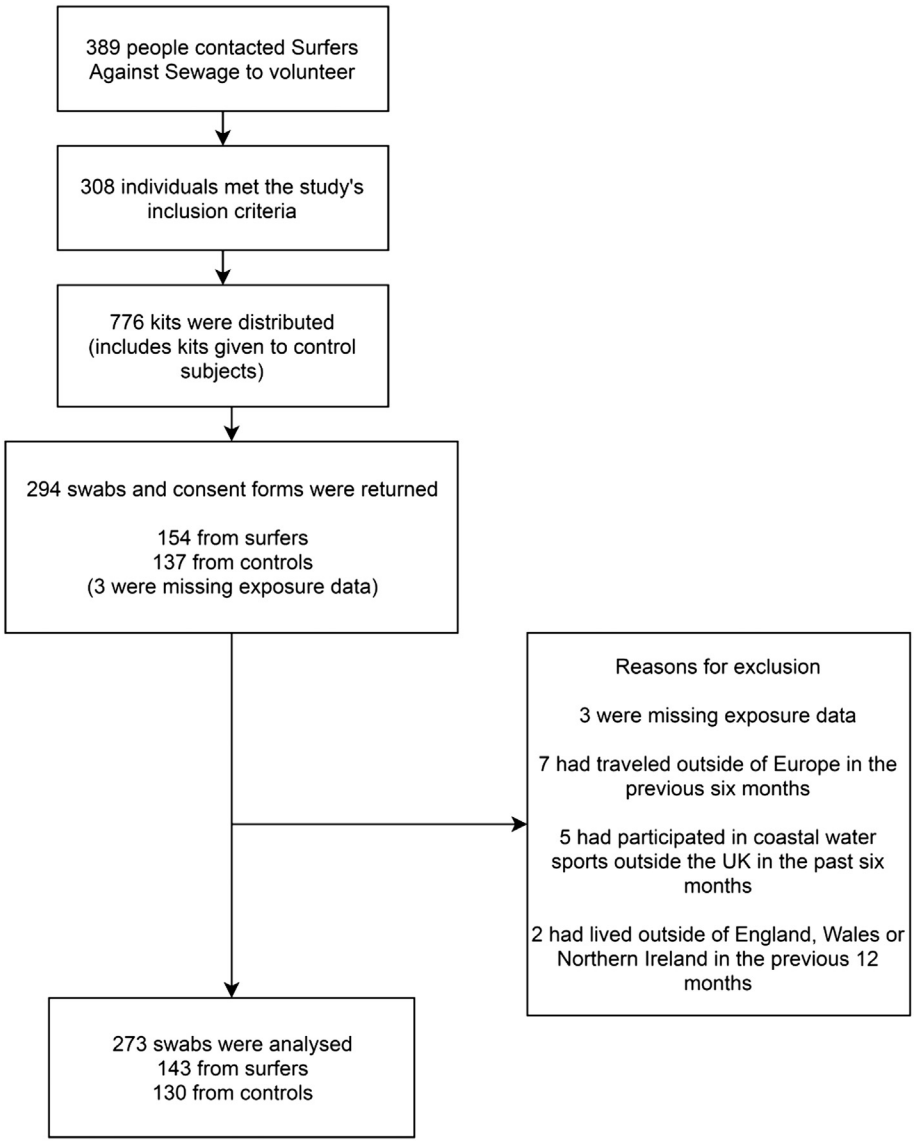

Fig. 2. Participant flow diagram and reasons for excluding participants.

Table 3
The number (%) of surfers and controls colonised by antibiotic-resistant *E. coli*.

	Surfers (N = 143)	Controls (N = 130)	Risk ratio (95% CI)	*p* value
Carriage of cefotaxime-resistant *E. coli*	13 (9.1%)	4 (3.1%)	2.95 (1.05 to 8.32)	0.040
Carriage of bla_{CTX-M}-bearing *E. coli*	9 (6.3%)	2 (1.5%)	4.09 (1.02 to 16.4)	0.046

bearing *E. coli* and were recruited into a cross-sectional survey to investigate whether surfers regularly using coastal waters are at an increased risk of colonisation by these ARB compared to individuals rarely exposed to coastal waters. In the Beach Bum Survey, an association was found between regular surfing and gut colonisation by cefotaxime-resistant *E. coli*. Surfers were approximately three times as likely as people who do not surf to be colonised by cefotaxime-resistant *E. coli* (risk ratio = 2.95, 95% CI 1.05 to 8.32, $p = 0.040$). Similarly, an important association was found between recreational use of coastal waters and gut colonisation by bla_{CTX-M}-bearing *E. coli*. People who regularly surf or body board were four times as likely to be colonised by *E. coli* harbouring this gene (risk ratio = 4.09, 95% CI 1.04 to 16.6, $p = 0.046$).

These findings are of public health significance: bla_{CTX-M} genes encode resistance to multiple antibiotics, such as cephalosporins, which have been classed as essential medicines by the World Health Organization as they are the "sole therapy or one of a few alternatives to treat serious human disease" (Collignon et al., 2009). Characterisation of the bla_{CTX-M}-bearing *E. coli* revealed of the 11 people colonised by bla_{CTX-M}-bearing *E. coli*, seven were colonised by *E. coli* O25b-ST131, a pathogenic strain of *E. coli* (Clermont et al., 2009; Rogers et al., 2011). Surfers were more than five times as likely as people who do not surf to be colonised by bla_{CTX-M}-bearing *E. coli* O25b-ST131, although this association had wide confidence intervals and was not statistically significant (risk ratio = 5.45, 95% CI 0.85 to 35.1, $p = 0.074$). Meropenem-resistant *Enterobacter cloacae* were also isolated from survey participants. *Enterobacter cloacae* is an opportunistic pathogen, causing serious infections in susceptible individuals (Davin-Regli and Pages, 2015). However, there is little evidence of an association between surfing and gut colonisation by meropenem-resistant bacteria.

While surfers colonised by potentially pathogenic ARB may by asymptomatic, gut bacteria are a major source of infection, and these bacteria may cause problems if colonised individuals develop a health condition in the future that makes them more susceptible to infections (Asir et al., 2015; Manaia, 2017). Furthermore, water users may be contributing to the overall carriage of ARB in the community, and may

Table 4
Characteristics of participants and their distribution among surfers and controls. Domestic risk includes any of: private water supply at home, pet ownership, a member of the household works in a healthcare setting or works with animals, a member of household has been hospitalised in the past 6 months, a member of the household has been on antibiotics in the past 6 months, a member of the household has spent time abroad in the past 6 months. Occupational risk includes participants working or volunteering in healthcare settings, or with animals.

Characteristics	Surfers $N = 143$	Controls $N = 130$
Male [n (%)]	98 (68.5%)	50 (38.5%)
Age [n (%)]		
18–24	39 (27.3%)	33 (25.4%)
25–34	38 (26.6%)	30 (23.1%)
35–44	50 (35.0%)	20 (15.4%)
45–54	6 (4.2%)	23 (17.7%)
55–64	7 (4.9%)	14 (10.8%)
≥ 65	3 (2.1%)	10 (7.7%)
White [n (%)]	141 (98.7%)	127 (97.7%)
Education [n (%)]		
1–4 GCSEs or equivalent qualifications	6 (4.2%)	2 (1.5%)
5 or more GCSEs or equivalent	5 (3.5%)	6 (4.6%)
Apprenticeship	3 (2.1%)	1 (0.8%)
2 or more A-levels or equivalent	31 (21.7%)	35 (26.9%)
Bachelor's degree or equivalent, higher	84 (58.7%)	73 (56.2%)
Other qualification	14 (9.8%)	13 (10%)
Country of residence [n (%)]		
England	92 (39.1%)	90 (40.9%)
Wales	14 (9.8%)	8 (6.2%)
Northern Ireland	17 (14.0%)	4 (3.1%)
Missing	*20 (14.0%)*	*28 (21.5%)*
Does water sports in non-coastal environments on a regular basis [n (%)]	82 (57.3%)	40 (30.8%)
Last time participant went in the sea in the UK		
Less than 7 days ago	92 (64.3%)	1 (0.77%)
In the past month	42 (29.4%)	12 (9.23%)
More than a month ago	9 (6.29%)	61 (46.9%)
More than a year ago	0 (0%)	56 (43.1%)
Number of times in the past 6 months participant has been in the sea		
Never	0 (0%)	55 (42.3%)
1–5 times	5 (3.5%)	58 (44.6%)
6–10 times	7 (4.9%)	8 (6.2%)
11–15 times	11 (7.7%)	4 (3.1%)
16–20 times	8 (5.6%)	1 (0.8%)
21–25 times	14 (9.8%)	1 (0.8%)
More than 25 times	97 (67.8%)	2 (1.5%)
Beach recreation (non-watersports) [n (%)]	135 (94.4%)	88 (67.7%)
Traveled within Europe in the past 6 months [n (%)]	41 (28.7%)	43 (33.1%)
Regularly consumes meat (pork, lamb, chicken, beef or seafood) [n (%)]	127 (88.8%)	124 (95.4%)
Regularly consumes salad [n (%)]	134 (93.7%)	121 (93.1%)
Domestic risk [n (%)]	103 (72.0%)	106 (81.5%)
Occupational risk [n (%)]	28 (19.6%)	19 (14.6%)

Table 5
Phylogenetic groups of bla$_{CTX-M}$-bearing *E. coli* isolated from survey participants, and *bla*$_{CTX-M}$ gene identity. Numbers in parentheses indicate the number of *bla*$_{CTX-M}$-bearing *E. coli* O25b-ST131 colonies identified.

E. coli phylogroup	Surfers colonised by *bla*$_{CTX-M}$-bearing *E. coli* ($N = 143$)		Controls colonised by *bla*$_{CTX-M}$-bearing *E. coli* ($N = 130$)
	CTX-M-15	CTX-M-57	CTX-M-15
A	0	0	0
B1	0	1	0
B2 (*E. coli* O25b-ST131)	6 (6)	0	1 (1)
C	1	0	0
D	0	0	1
E	0	0	0
F	1	0	0
Total	8	1	2

spread ARB among the wider population (Valverde et al., 2008). A higher community carriage of ARB puts vulnerable people (such as immunocompromised people) at increased risk of acquiring ARB. This constitutes a serious threat to the health of these susceptible individuals, who may subsequently develop drug-resistant infections.

The prevalence of colonisation by *bla*$_{CTX-M}$-bearing *E. coli* in the epidemiological survey (6.3% and 1.5% in surfers and controls respectively) is lower than the 11.1% recently reported in a UK community (Wickramasinghe et al., 2012). This is probably due to the eligibility criteria set for this study, which excluded people who were likely to have been colonised in other environments. In addition, a large number of survey participants lived in the Southwest of England (Table S7), where there are lower population densities and less immigration; factors which are thought to impede the spread of ARB among members of rural communities (Office for National Statistics, 2013; Rienzo and Vargas-Silva, 2014; Wickramasinghe et al., 2012). Despite the low prevalence of colonisation by resistant *E. coli* in the epidemiological survey, the risk is still three times higher in bathers compared to non-bathers, and the risk ratios presented are greater than those reported in previous studies specifically investigating acquisition of ESBL-producing *Enterobacteriaceae* in healthcare settings (Ebrahimi et al., 2014; Munday et al., 2004; Valverde et al., 2008). This suggests that the risk of colonisation by ARB associated with exposure to natural environments could be more important than previously thought. There are likely many different ways people can be exposed to ARB (such as by eating contaminated food), so understanding the various means by which people are exposed to and colonised by ARB, as well as the risk magnitude and the number of people at risk will allow the formulation of effective strategies to reduce the spread of ARB and may have implications for the way we consider community acquisition of ARB.

The results of environmental monitoring (Section 3.1) are likely to be an underestimate of the proportion of resistant bacteria and resistance genes in coastal environments (Leonard et al., 2015). One group of culturable bacteria resistant to two clinically important antibiotics was investigated, although resistance among non-*E. coli* coliforms isolated from bathing waters and Beach Bum Survey participants was also examined (Tables S4 and S6). While the proportion of bathing water *E. coli* and non-*E. coli* coliforms harbouring *bla*$_{CTX-M}$ was the same, among the coliforms isolated from Beach Bum Survey participants, only *E. coli* were found to harbour *bla*$_{CTX-M}$ (Table S6). As anticipated *bla*$_{CTX-M-15}$ was the most common genotype identified in *E. coli* from both bathing waters and survey participants (Hawkey and Jones, 2009). A rarer genotype, *bla*$_{CTX-M-57}$, was identified in one surfer, which was not found in bathing waters, and *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-27}$ were found in bathing waters but not in survey participants. These observations could be explained by the fact the environmental monitoring was done three years prior to the Beach Bum Survey, and the diversity of *bla*$_{CTX-M}$ borne by *E. coli* in bathing waters could have changed. Additionally, the diversity of *bla*$_{CTX-M}$ in bathing waters was based upon fewer than 100 samples, which might not have captured the extent of genetic diversity in this environment.

Participants were not screened for their colonisation status prior to surfing, and therefore it is uncertain that surfers acquired their ARB from coastal waters. However, by excluding people who had likely been exposed in other environments (see Appendix for eligibility criteria), other known sources of contamination were minimized, as well as ensuring that people with infections severe enough to require hospitalisation or treatment with antibiotics were excluded. There were noticeable differences in the age and sex of participants between surfers and non-surfing control subjects (Table 4). However, while surfers were more likely to be male and younger than 45, there was no association between sex or age and colonisation by *bla*$_{CTX-M}$-bearing *E. coli*. Other potential confounders, like diet, were also investigated for their association with the exposure and outcome under investigation, but none were found to be confounders. It possible that unmeasured confounders (such as whether participants were experiencing minor illnesses), and

systematic differences between survey respondents and non-respondents might affect participants' exposure to ARB or susceptibility to colonisation by ARB. Despite the wide confidence intervals surrounding the risk ratio estimates, the lower limits are still above the null value, indicating an overall increase in risk to UK surfers of gut colonisation by resistant *E. coli*. The risk of colonisation by ARB among surfers might be different in other countries. For example, in countries with tropical climates and warmer waters, surfers might typically be exposed much more frequently than 3 times per month. Similarly, in places where people swim in the sea more often, the risk might be substantially higher than the risks reported in the UK and other countries with temperate climates.

There are numerous and complex interactions at play between humans, ARB reservoirs, and ARB, affecting the survival, dispersal, infectivity, pathogenicity and persistence of ARB (Ashbolt et al., 2013; Manaia, 2017). Further research is essential in order to understand these interactions and their impact upon public health. Coastal waters represents one compartment of many natural and non-hospital environments, which all require further investigation of their role in the transmission of ARB. The clinical significance of higher rates of colonisation is suspected to be important based on current evidence, but are yet to be confirmed in surfers.

5. Conclusions

For the first time, the risk of exposure to *E. coli* harbouring mobile $bla_{CTX\text{-}M}$ genes in coastal waters has been quantified. Furthermore, this is the first study to identify an association between surfing and gut colonisation by antibiotic-resistant *E. coli*. Further work must be done to establish the acquisition of ARB from coastal waters and other natural environments which have been identified as important reservoirs of ARB.

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Conflicts of interest

Professor Hawkey reports grants and personal fees from Eumedica, grants and personal fees from Pfizer, personal fees from Merck, personal fees from Novartis, personal fees from Magus Communications, personal fees from Bio Merieux, personal fees from Wyth, from Becton-Dickinson, personal fees from Novacta, personal fees from Novolytics, outside the submitted work. Dr. Gaze reports grants from Medical Research Council, grants from Biotechnology and Biological Sciences Research Council, grants from AstraZeneca, outside the submitted work. The authors have no other financial conflicts of interest to declare.

Data access statement

The environmental surveillance data supporting this publication can be publicly accessed in Open Research Exeter via the following persistent identifier: http://hdl.handle.net/10871/30448. Due to ethical concerns, the epidemiological data are not publicly available.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2017.11.003.

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