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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 25 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