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The potential human exposure to antibiotic resistant-*Escherichia coli* through recreational water

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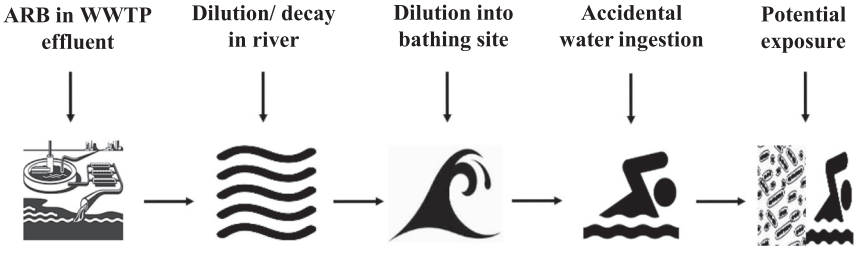
HIGHLIGHTS

- Human exposure to antibiotic resistant bacteria through bathing water was examined.
- Impact of nearby wastewater treatment plants on bathing water sites was analysed.
- The mean human exposure levels ranged between 0.45 and 345.09 cfu/100 ml.
- This study provides valuable information for regulatory bodies and policy makers.

GRAPHICAL ABSTRACT



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ABSTRACT

It is important that bathing water sites are free as possible from antibiotic resistant bacteria (ARB) to prevent the spread of difficult to treat infections throughout the population. This study examines the possible human exposure to antibiotic resistant *Escherichia coli* (AR-*E. coli*) through recreational activities at two different bathing water sites located near wastewater treatment plants (WWTPs). A quantitative risk assessment model was created to model the pathway of the AR-*E. coli* from the WWTPs effluent water through to the bathing water sites. Both sampling data and data from scientific literature were used. The main steps considered for the model were: the dilution and decay of the AR-*E. coli* from the WWTPs effluent water into the river; the dilution of the river into the bathing water sites and the human exposure to AR-*E. coli* through recreational activities at the bathing water sites (as a result of water ingestion). The results show the mean predicted human exposure levels ranged between 0.45 and 345.09 cfu/100 ml. A back calculation method determined that in accordance with the European Bathing Water Directive (2006/7/EC) (BWD) to be considered "poor" water quality, the concentration of AR-*E. coli* in WWTP effluent water would need to exceed 2.45 log cfu/ml at site 1 and exceed 2.71 log cfu/ml at site 2. This study provides valuable information for regulatory bodies and policy makers on the possible human exposure levels to AR-*E. coli* and the maximum permissible concentrations in WWTP effluent water to ensure compliance with relevant bathing water legislation.

1. Introduction

Microbial contamination is a common problem associated with bathing water sites, leading to beach closures and a loss of tourism when contamination events occur (Hamilton et al., 2010). Pathogenic agents found in bathing water are often resulting from fecal pollution

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caused by inadequately treated sewage from wastewater treatment plants (WWTPs), storm water discharges, runoff from agricultural activities, inflow from contaminated rivers, discharges from ships, wildlife or domestic animals (Riou et al., 2007; Abdelzaher et al., 2010; Fewtrell and Kay, 2015). The most frequently acquired illness as a result of exposure to contaminated bathing water is gastrointestinal illness (GI) (Fewtrell and Kay, 2015; European Environment Agency, 2017). It is estimated that swimming and bathing at wastewater contaminated water sites leads to 120 million cases of GI globally (Shuval, 2003). Also respiratory illness, which can be a more serious health outcome, are estimated at >50 million cases from swimming and bathing at water sites impacted by wastewater (Shuval, 2003; WHO, 2003; Mannocci et al., 2016).

Water quality guidelines to estimate the health risk to swimmers from bathing water have been developed based on the concentrations of indicator bacteria e.g. *Escherichia coli* (Roser et al., 2006; Abdelzaher et al., 2010). Recent evidence suggest that WWTPs may promote the development of antibiotic resistance (AR) in the microbial community of the receiving waters (Harris et al., 2012; Karkman et al., 2018; Manaia et al., 2018; O'Flaherty et al., 2018a) Additionally, several authors showed the presence of AR-*E. coli* in recreational water around the world (Turgeon et al., 2012; Fernandes et al., 2017; Mahon et al., 2017; Leonard et al., 2018). Bathers and swimmers using contaminated bathing water sites could become colonized by AR-*E. coli*, this could lead to the spread of antibiotic resistance (AR) in the community (Leonard et al., 2018). The colonization of humans with AR-*E. coli* may not always be an immediate human health threat, however, it can lead to bacterial infections that have a higher risk of treatment failure and the potential for AR to spread to other bacterial strains (Hammerum and Heuer, 2009).

Human exposure assessment models are valuable methods that have been used to investigate the risk of human exposure to harmful bacteria through bathing water (Dorevitch et al., 2011; Schets et al., 2011). In particular, the use of predictive modeling techniques to estimate possible concentrations of *E. coli* pollution in bathing water is a good alternative to the traditional water monitoring which requires labored and time consuming effort (Nevers et al., 2007). Risk assessments examining the human exposure to AR-*E. coli* through bathing water could help to inform regulatory bodies about the adequate management of a beach (Eregno et al., 2016). Additionally, recreational water maybe a critical pathway where human exposure to ARB can occur, however, little research has been done on the potential significance that bathing water can play in the spread of ARB in the community (Turgeon et al., 2012; Leonard et al., 2015). Even though *E. coli* contamination is tested for routinely at bathing sites, AR-*E. coli* contamination is not tested for and there is a lack of data on the concentrations of AR-*E. coli* at bathing water sites (Eregno et al., 2016).This study examines the potential human exposure to single antibiotic resistant *E. coli* (SAR) (resistant to at least one antibiotic), multi-drug resistant *E. coli* (MDR) (resistant to 3 or more antibiotics of different classes) and potentially pathogenic antibiotic resistant *E. coli* (PAR) (resistant to at least one antibiotic) through recreational activities at two Italian bathing sites located nearby WWTPs. A quantitative human exposure assessment model was created to estimate the potential human exposure to AR-*E. coli* through bathing water and the concentration of AR-*E. coli* required in the nearby WWTPs effluent water in order for the BWD to be exceeded was also examined.

2. Material and methods

2.1. Model structure and development

A human exposure assessment model was created for this study to investigate the human exposure to AR-*E. coli* through two bathing water sites. The main steps involved in the development and structure of the model are shown in Fig. 1. The first step of the model was to analyse the observed concentrations AR-*E. coli* found in the WWTP effluent water and in the bathing water at the two study sites (Table 1). Using the AR-*E. coli* concentrations tested in the WWTP effluent water, the dilution of the WWTP effluent water into the river was calculated to estimate the concentration of AR-*E. coli* in the mixed water of the river. The decay and effect of environmental factors on the concentration of AR-*E. coli* in the river was modelled. The dilution of the river into the bathing water site was calculated to get the predicted concentration of AR-*E. coli* at the bathing water sites. The amount of water ingested by beach goers was estimated through a beach survey and scientific literature. The human exposure to AR-*E. coli* through recreational water (ingestion of water) was then estimated by multiplying the predicted concentration of AR-*E. coli* in the bathing water by the amount of water consumed by beach goers.

2.2. AR-E. coli sampling at WWTP and bathing sites

For this study two rivers located near Rome (Central Italy) were selected, both rivers have a WWTP and a recreational beach not far from their river mouths (approximately 3 km away). Three replicate 1 l water samples were collected from the WWTP effluent waters located on the River Arrone and Tiber respectively, and from the near shore waters of Ostia and Fregene beaches in July and late August 2016 (N = 24). Samples after serial dilutions were filtered and analysed in the lab for the isolation, identification and enumeration of AR-*E. coli* after cultivation on Tryptone Bile X-Glucuronide Medium (TBX, Oxoid, Cambridge, UK) and overnight incubation at 37 °C. The isolates were then confirmed as *E. coli* with the API-20 E system (Biomérieux, France). Phenotypic resistance to antibiotics on 30 randomly selected *E. coli* colonies per growing plate was tested through the Kirby-Bauer disk diffusion technique (Bauer et al., 1966) following EUCAST (2017). Selected antibiotics and concentration were: Tetracycline (16 μg/ml), Imipenem (10 μg/ml), Chloramphenicol (30 μg/ml), Ciprofloxacin (5 μg/ml), Trimethoprim-Sulfamethoxazole (64 μg/ml), Amoxicillin (2 μg/ml), AUG2 (Amoxicillin/clavulanic acid; 20/10 μg/ml), Gentamicin (10 μg/ml), Cefotaxime (5 μg/ml). Additionally, *E. coli* isolates were tested by end-point PCR for the presence of virulence genes associated with *E. coli* pathotypes: Enterotoxigenic (ETEC causes infectious diarrhea by producing heat-stable and/or heat-labile enterotoxins that target the intestinal mucosa); Enteropathogenic (EPEC is commonly associated with infantile diarrhea and is caused by the bacteria attaching tightly to the host cell membrane and disrupting the cell surface leading to effacement of microvilli); Enteroinvasive (EIEC causes blood, mucus, and leukocytes in stools caused by the bacteria invading the human colonic mucosa); Enterohaemorrhagic (EHEC, causes diarrhea and hemolytic uremic syndrome by producing shiga toxin that binds to endothelial cells and absorbs into the bloodstream) and Enteroaggregative (EAEC causes acute and persistent diarrheal disease through the bacteria adhering to the intestinal cells and producing enterotoxins and cytotoxins) (Okhuysen and Dupont, 2010; Ochoa and Contreras, 2011; Nguyen and Sperandio, 2012; von Mentzer et al., 2014; Pasqua et al., 2017). No ETEC or EIEC virulence factors were found in any isolate. *E. coli* isolates were arranged into three categories to examine single antibiotic resistant *E. coli* (SAR) (resistant to at least one antibiotic), multi-drug resistant *E. coli* (MDR) (resistant to 3 or more antibiotic of different classes) and potentially pathogenic antibiotic resistant *E. coli* (PAR) (resistant to at least one antibiotic and tested positive for at least one virulence factor of EHEC, EPEC or EAEC pathotypes). Best fit probability distributions were used to characterise the concentrations of AR-*E. coli* found in the WWTPs effluent water and at the bathing sites of the two study sites (Table 1).

2.3. AR-E. coli dilution and decay in river

There are two main factors to consider when modeling the dispersion and survival of bacteria in water: they are the physical dilution