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Prevalence and characteristics of ESBL-producing *E. coli* in Dutch recreational waters influenced by wastewater treatment plants

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ABSTRACT

Outside health care settings, people may acquire ESBL-producing bacteria through different exposure routes, including contact with human or animal carriers or consumption of contaminated food. However, contact with faecally contaminated surface water may also represent a possible exposure route. The current study investigated the prevalence and characteristics of ESBL-producing *Escherichia coli* in four Dutch recreational waters and the possible role of nearby waste water treatment plants (WWTP) as contamination source. Isolates from recreational waters were compared with isolates from WWTP effluents, from surface water upstream of the WWTPs, at WWTP discharge points, and in connecting water bodies not influenced by the studied WWTPs. ESBL-producing *E. coli* were detected in all four recreational waters, with an average concentration of 1.3 colony forming units/100 ml, and in 62% of all samples. In surface waters not influenced by the studied WWTPs, ESBL-producing *E. coli* were detected in similar concentrations, indicating the existence of additional ESBL-*E. coli* contamination sources. Isolates with identical ESBL-genes, phylogenetic background, antibiotic resistance profiles, and sequence type, were obtained from effluent and different surface water sites in the same watershed, on the same day; occasionally this included isolates from recreational waters.

Recreational waters were identified as a potential exposure source of ESBL-producing *E. coli*. WWTPs were shown to contribute to the presence of these bacteria in surface waters, but other (yet unidentified) sources likely co-contribute.

1. Introduction

During the last two decennia, the prevalence of Extended Spectrum Beta-Lactamase (ESBL)-producing bacteria has increased worldwide (Cantón et al., 2008; Castanheira et al., 2008). ESBL-producing bacteria are resistant to most beta-lactam antibiotics, including 3rd and 4th generation cephalosporins, and are often additionally resistant to multiple other classes of antibiotics. This severely limits treatment options for infections caused by these bacteria, which has led to an increased use of last-resort antibiotics such as carbapenems (Cantón et al., 2012). Although initially

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ESBL-production was typically associated with hospital-acquired infections caused by *Klebsiella pneumoniae*, it is now also associated with community-acquired infections, mainly urinary tract infections caused by *Escherichia coli* (Livermore et al., 2007; Paterson and Bonomo, 2005). Moreover, ESBL-producing *E. coli* are present among the commensal *E. coli* population in healthy individuals and food-producing animals (Huijbers et al., 2013; Nethmap_-MARAN, 2013; Trott, 2013). Commensal *E. coli* generally do not cause disease, however, spread of ESBL-producing variants through human and animal populations is nevertheless worrisome, and may lead to increased exposure of populations more susceptible to opportunistic infections (e.g. the elderly or hospitalized individuals). Additionally, with an increasing number of ESBL-producing *E. coli* carriers in the human population the risk increases that gut pathogens efficiently acquire resistance by gene transfer of ESBL-genes as well as other antibiotic resistance genes from ESBL-producing *E. coli* in the intestinal tract.

Dissemination of ESBL-producing *E. coli* outside the health care setting may be facilitated by contact with human or animal carriers, or consumption of contaminated animal products. Additionally, a possible role for the environment should be considered in this regard. Since *E. coli* are commensal bacteria, they are abundantly excreted into the environment, amongst others through application of manure as fertilizer or droppings of pasture animals, with feces of wild animals, and with discharge of (partially) treated wastewater, or with sewage overflows during heavy rainfall. Some of the commensal as well as pathogenic *E. coli* that are excreted into the environment may have the capacity to produce ESBL. Indeed, ESBL-producing *E. coli* have been detected in surface water worldwide, including the Netherlands (Blaak et al., 2011; Chen et al., 2010; Dhanji et al., 2011; Hong et al., 2004). Human exposure to these bacteria may occur, for instance during recreation in contaminated surface water, or indirectly, when contaminated surface water is used for irrigation of (raw consumed) crops, therewith contributing to community-associated dissemination of ESBL-producing *E. coli*. Additionally, contaminated surface water might contribute to exposure of animals (wild life as well as livestock) that drink from it.

In order to limit dissemination of ESBL-producing *E. coli* through the environment, insight in the contribution of different possible environmental contamination sources and exposure routes is essential. The current study determined the presence of ESBL-producing *E. coli* in four Dutch recreational water regions, and the possible contribution of nearby wastewater treatment plants.

2. Materials and methods

2.1. Sampling and sampling locations

Three recreational waters appointed under European Bathing Water Directive 2006/7/EC ('official') (Anonymous, 2006) and one not appointed ('unofficial') recreational water were sampled during the bathing seasons of 2011 and/or 2012 which, in the Netherlands, lasts from May 1st until September 30th. The recreational waters were situated in different regions in the Netherlands, and each was located 1–2 km (as the crow flies) from a wastewater treatment plant (WWTP) that did not disinfect treated effluents before discharge (Fig. 1). Two of the official recreational waters (regions A and B) were situated in freshwater lakes; the third (region C) was located in the

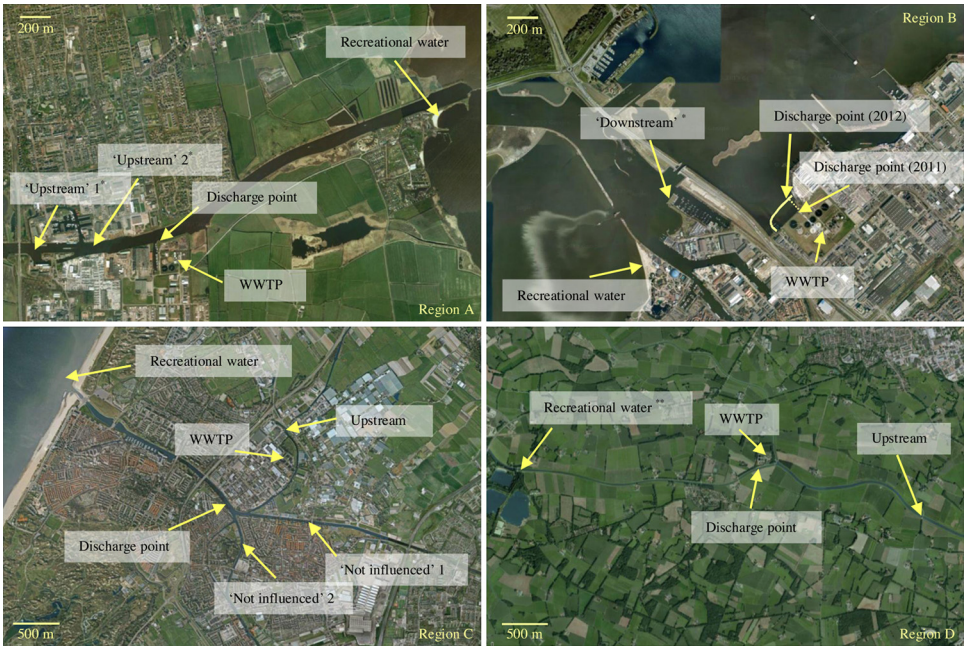

Fig. 1. Sampling locations in regions A, B, C, and D. *For regions A and B, the direction of currents is variable and 'upstream' and 'downstream' annotations are based on the main current direction. ** The entire river is used for recreation, the indicated site is chosen to represent recreational water downstream of an WWTP.