Antibiotic resistance along an urban river impacted by treated wastewaters

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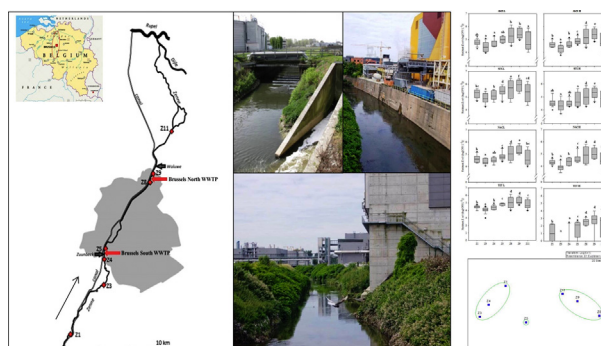[c] *Group of Molecular Microbial Ecology, Institute of Aquatic Ecology, University of Girona, Girona, Spain*
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HIGHLIGHTS

- Antibiotic resistant (AR) *E.coli* increased downstream the release of WWTP effluents.
- Significant regression between AR *E. coli* and AR heterotrophic bacteria was found.
- Tetracycline concentration significantly correlated with respective ARGs abundance.
- Particle-attached bacteria showed higher levels of some ARGs than free-living ones.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Urban rivers are impacted ecosystems which may play an important role as reservoirs for antibiotic-resistant (AR) bacteria. The main objective of this study was to describe the prevalence of antibiotic resistance along a sewage-polluted urban river. Seven sites along the Zenne River (Belgium) were selected to study the prevalence of AR *Escherichia coli* and freshwater bacteria over a 1-year period. Culture-dependent methods were used to estimate *E. coli* and heterotrophic bacteria resistant to amoxicillin, sulfamethoxazole, nalidixic acid and tetracycline. The concentrations of these four antibiotics have been quantified in the studied river. The antibiotic resistance genes (ARGs), *sul*1, *sul*2, *tet*W, *tet*O, *bla*TEM and *qnr*S were also quantified in both particle-attached (PAB) and free-living (FLB) bacteria. Our results showed an effect of treated wastewaters release on the spread of antibiotic resistance along the river. Although an increase in the abundance of both AR *E. coli* and resistant heterotrophic bacteria was observed from upstream to downstream sites, the differences were only significant for AR *E. coli*. A significant positive regression was also found between AR *E. coli* and resistant heterotrophic bacteria. The concentration of ARGs increased from upstream to downstream sites for both particle-attached (PAB) and free-living bacteria (FLB). Particularly, a significant increase in the abundance of four among six ARGs analyzed was observed after crossing urban area. Although concentrations of tetracycline significantly correlated with tetracycline resistance genes, the antibiotic levels were likely too low to explain this correlation. The analysis of ARGs in different fractions revealed a significantly higher abundance in PAB compared to FLB for tetO and sul2

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genes. This study demonstrated that urban activities may increase the spread of antibiotic resistance even in an already impacted river.

1. Introduction

Indiscriminate use and overuse of antibiotics has led to an increase in the prevalence of antibiotic-resistant (AR) bacteria (Levy and Marshall, 2004). The use of antimicrobial agents and their subsequent release in aquatic environments may have consequences for autochthonous bacterial communities, especially in freshwater ecosystems. The direct effects of antibiotics can be detrimental to the ecosystem since autochthonous bacteria play key roles in biogeochemical processes (Costanzo et al., 2005). Moreover, recent studies have revealed that sub-inhibitory antibiotic concentrations, similar to those found in some aquatic environments (Kümmerer, 2009), may promote selection of AR bacteria (Gullberg et al., 2011). In addition, AR determinants may be considered as a form of pollution in sewage-impacted rivers (Martinez, 2009) given that they are introduced into the environment mainly by the release of enteric bacteria (Alonso et al., 2001).

During periods of treatment with antibiotics, bacteria from gastrointestinal tract are exposed to high concentrations of those compounds and develop resistance therein before being released into the aquatic environment, through treated or untreated wastewater, surface runoff and soil leaching (Servais and Passerat, 2009). Urban rivers are of the most involved environments, receiving both antibiotics and AR fecal bacteria from wastewater treatment plant (WWTP) effluents. Different studies reported the presence of AR opportunistic pathogens (Vancomycin-Resistant Enterococci, *Klebsiella pneumoniae*, *Acinetobacter*, *Psuedomonas* spp. and *Shigella* spp.) in urban rivers affected by treated and untreated wastewaters (Hladicz et al., 2017; Marathe et al., 2017; Nishiyama et al., 2017; Skariyachan et al., 2015). In general, these impacted ecosystems play an important role in driving the persistence and spread of AR bacteria (Taylor et al., 2011). In fact, urban rivers provide a setting in which the horizontal exchange of mobile genetic elements encoding antibiotic resistance between fecal and freshwater bacteria can take place (Zhang et al., 2009). It is therefore of major importance to investigate the main drivers of resistance behavior in freshwater bacteria to identify possible management strategies able to control and reduce the dissemination of antibiotic resistance in bacterial communities of freshwater environments.

Many works investigated the behavior of antibiotic residuals in freshwaters (Gibs et al., 2013; Kümmerer, 2009; Zuccato et al., 2010) whereas many others focused on the ARGs prevalence along sewage impacted rivers (Devarajan et al., 2016; Pruden et al., 2012; Stoll et al., 2012). Moreover, several studies aimed to analyze the occurrence and fate of antibiotic-resistant bacteria (ARB) in aquatic environments affected by WWTPs release (Alm et al., 2014; Garcia-Armisen et al., 2013; Souissi et al., 2018). Despite considerable amount of research have been carried out coupling the investigation of antibiotics and ARGs behavior (Huerta et al., 2013; Khan et al., 2013; Rodriguez-Mozaz et al., 2015) and that of ARB and ARGs (Guyomard-Rabenirina et al., 2017; Zhang et al., 2014), comprehensive studies assessing at the same time the fate of antibiotics, ARB and ARGs in urban rivers affected by wastewaters are still lacking. One original study investigated the relationship between antibiotics, ARB and ARGs in waters along a medical center—WWTP—river continuum (Oberlé et al., 2012). Nevertheless, this study only considered the fecal indicator *E.coli* and mainly focused on the sewage treatment system only sampling the river upstream from the release of the WWTP effluent (Oberlé et al., 2012).

The main objective of this study was then to describe the occurrence of antibiotic pollution and the prevalence of ARB along a sewage-impacted urban river, focusing on *Escherichia coli,* freshwater bacterial communities and ARGs. In the present study, *E. coli* is used as a model of bacteria from enteric origin. We choose to use *E. coli* for such a model as it is the most widely used fecal indicator bacteria to evaluate the level of recent microbiological contamination in waters (Edberg et al., 2000). Enteric bacteria can be exposed to high antibiotic concentrations in the human or animal gastrointestinal tract and could acquire resistance before being released in the environment. These bacteria can thus act as a source of resistance in natural environments because they can disseminate antibiotic resistance genes (ARGs) to freshwater bacteria (Davison, 1999). Considering that low antibiotic concentrations (lower than minimal inhibitory concentration) are able to promote antibiotic resistance (Gullberg et al., 2011), continuous release of low levels of antibiotics in river water could act as chronic selective pressure on freshwater bacterial communities possibly contributing to the spread of resistance in aquatic environments.

To investigate the AR spread along a sewage impacted river, the Zenne River was studied. The Zenne is a paradigm of sewage-impacted river because its discharge (on annual average) is doubled after receiving the treated waters from the two WWTPs in the city of Brussels (Brion et al., 2015); high levels of fecal contamination have been already described in this river (Ouattara et al., 2014). Seven sites along the Zenne River were sampled for 1 year to study the prevalence of AR *E. coli* and freshwater bacteria, particularly focusing on the influence of treated sewage waters on the AR behavior along the watercourse. Culture-dependent and -independent methods were used to estimate the resistance of *E. coli* and heterotrophic bacteria by plate counts containing or not containing antibiotics as well as by quantifying the abundance of six genes conferring resistance to the main antibiotic families in both particle-attached (PAB) and free-living (FLB) bacteria. We hypothesized that after the release of sewage waters into the river the amount of resistant *E. coli* isolates would increase and that this increase would be reflected on the freshwater bacteria and on the river resistome. Moreover, it was expected to find higher levels of ARGs on PAB with respect to FLB because close contact between cells attached to the same particle would increase the probability of exchange of genetic material encoding resistance.

2. Material and methods

2.1. Study site and sampling strategy

The Zenne River is located in the Belgian part of the Scheldt watershed and is a tributary of the Dijle River (Fig. 1). The Zenne watershed (991 km^2) is characterized by agricultural activities in its upstream part and urbanization downstream. The population density in the watershed is on average 1260 inhabitants per km^2 and mostly located in Brussels region. The Zenne has a length of 103 km and crosses Brussels from south to north over a distance of about 20 km. Its annual average discharge upstream from Brussels is 4 m^3 s^{-1} (Brion et al., 2015). Before the river reaches the Brussels area, it already receives several effluents from small-scale WWTPs. In the Brussels area, the Zenne receives effluents from two large WWTPs: the Brussels South WWTP (360,000 equivalent-inhabitants) and the Brussels North WWTP (1.2 million equivalent-inhabitants). The Brussels South WWTP treatment line includes a primary settling stage and a secondary biological treatment (activated sludge). At the Brussels North WWTP the treatment includes a primary settling stage followed by a modern tertiary treatment