

## Illicit Connections and Antibiotic Resistant Bacteria in Urban Surface Water

La relation entre branchements illicites et bactéries résistantes aux antibiotiques dans les eaux de surface urbaines

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### RÉSUMÉ

Les bactéries résistantes aux antibiotiques représentent une menace pour la santé humaine. Elles peuvent être transmises par les eaux de surface contaminées. Les effluents des STEP, les débordements d'égouts unitaires et le ruissellement des terres agricoles figurent parmi les sources connues de contamination. Cette étude révèle la présence très répandue de *E. coli* et d'*Enterococcus* spp. résistantes à l'ampicilline et productrices de BLSE dans les eaux de surface urbaines des Pays-Bas, qui ne font pas partie de ces sources connues. Les auteurs étudient la relation possible entre ces bactéries présentes dans les eaux de surface et les branchements illicites sur les égouts pluviaux qui se déversent ensuite dans les eaux de surface. L'effet des branchements illicites est évalué par échantillonnage avant et après leur localisation et leur suppression dans un bassin versant urbain de la ville d'Almere, aux Pays-Bas.

### ABSTRACT

Antibiotic resistant bacteria pose a threat to human health care. People may acquire such bacteria through contaminated surface waters. Known sources of contamination are WWTP effluent, combined sewer overflows (CSO) and run-off from agricultural lands. This study shows the wide-spread presence of ESBL-producing *E.coli* and ampicillin-resistant *Enterococcus* spp. in urban surface waters in the Netherlands that are not subject to these known sources. It studies the possible relation between these bacteria in surface water and any illicit connections in the storm sewers that discharge to the surface waters. The effect of illicit connections is assessed by sampling prior to and after the localization and removal of illicit connections in an urban catchment area in the municipality of Almere, the Netherlands.

### KEYWORDS

Antibiotic Resistant Bacteria; Surface Water Quality; Illicit Connections, DTS

## 1 INTRODUCTION

Over the last decades, the prevalence of antibiotic resistant bacteria such as Extended Spectrum Beta Lactamase (ESBL)-producing Enterobacteriaceae has increased throughout Europe (Cantón et al., 2008). The current number of carriers in the Netherlands is estimated at 5% of the population. This poses a threat to human health care as infections caused by these bacteria are generally difficult to treat and require the use of last-resort antibiotics.

Several exposure routes for humans to antibiotic resistant bacteria are known, such as within health care settings, through contact with animal carriers (e.g. on farms) and through the consumption of contaminated foods. Increasingly, contact with faecally contaminated surface water, e.g. during recreation, is considered another possible exposure route. An important contributor of faecal bacteria to surface waters is the urban wastewater system via two sources: WWTP effluent and combined sewer overflows (CSO). Estimations indicate that in the Netherlands both sources contribute a significant and roughly equal number of (resistant) bacteria to Dutch surface waters (STOWA, 2018).

In earlier work (Blaak et al., 2017) we demonstrated that antibiotic resistant bacteria can also be present in urban surface waters in the Netherlands that are not under the influence of WWTP effluent or CSOs. One example was the surface water of a hydraulically isolated, 2300 ha urban catchment area in the municipality of Almere with around 85.000 inhabitants. The area was selected because of the complete absence of WWTPs and combined sewers discharging to the water system; instead, the area has separate sewer systems only. A sampling campaign in the summer of 2016 from 15 randomly selected locations during both wet and dry weather showed the abundant presence of ESBL-EC (a resistant variant of E.Coli) and ARE (a resistant variant of Enterococcus spp.) in the catchment area. In total, ESBL-EC was found in 17 samples (57%) and ARE in 11 samples (37%).

Without WWTP effluent, CSOs and agricultural influences, the source(s) of these faecal bacteria and their resistant variants must be local. A possible source is human wastewater discharged to the surface water system via storm sewers due to illicit connections. On the one hand, this would be in line with the facts that (1) the bacterial concentrations in surface water increased significantly during wet weather and (2) illicit connections are a common phenomenon associated with separate sewer systems. On the other hand, the number of locations and, in some cases, the measured concentrations seem rather high compared to expectations. Based on an ESBL-EC prevalence of 5% among the common population and an average of 2% illicit connections in the Netherlands, 'only' 85 out of the 85.000 inhabitants are expected to discharge ESBL-EC to the local Almere surface water system via their wastewater. It seems improbable that this relatively small volume of wastewater can lead to the 57% positive samples with a widespread geographical distribution. Possible explanations for this anomaly are (1) a higher ESBL-EC prevalence in the catchment, (2) more than 2% illicit connections in the area, and/or (3) other sources of (resistant) bacteria such as animal excrements in storm water run-off (birds, dogs, etc.) and excrements of waterfowl (ducks, geese and swans) in surface waters. Based on the 2016 results, no conclusions could be drawn on the origin of the (resistant) bacteria in the studied surface waters.

This extended abstract presents follow-up research that intends to address this anomaly and study more in depth the possible relation between illicit connections and the presence of antibiotic resistant bacteria in surface waters. The set up of the experiments has been presented at UDM 2018. However, due to a delay in the project, no results could be shown at the time. In this contribution to NOVATECH2019, the results will be available.

## 2 MATERIALS AND METHODS

### 2.1 Study approach

To (further) study the relation between illicit connections and bacterial contamination of surface water we use the following approach. In a selected catchment area we:

1. applied random sampling in local surface water and in the storm sewer (December 2017);
2. investigated the presence and locations of illicit connections (April/May 2018);
3. sampled the water, biofilm and sediment inside the storm sewer at and around the locations of illicit connections (May 2018);
4. remove all illicit connections from the storm sewer and clean the storm sewer (ongoing since October 2018, will be finished February 2019);

5. repeat the sampling in local surface water and in the storm sewer (March 2019).

Comparison of the sampling results of (1) and (5) will give an indication of the overall effect of the removal of illicit connections on the (bacterial) water quality in the storm sewer and in surface water. The sampling results of (3) give a more detailed view on the fate and spread of bacteria around illicit connections.

## 2.2 Research area

The catchment area selected for the study is a 100 ha residential area (Regenboogbuurt) in the municipality of Almere with around 5.400 inhabitants. The area has a separate sewer system with all storm sewers discharging to two central ponds in the neighbourhood, see Figure 1. The ponds are not interconnected, and discharge in opposite directions. This gives the possibility to study two independent, hydraulically isolated surface water systems that are not influenced by WWTP effluent, CSOs and agriculture.



Figure 1. Regenboogbuurt in the municipality of Almere with two central ponds receiving storm water (left); aerial view of one of the central ponds (right)

## 2.3 Methods

Each sample is analysed for the fecal bacteria *Escherichia coli* (*E.coli*) and *Enterococcus* spp. as well as the antibiotic resistant variations “Extended-Spectrum Beta-Lactamase”-producing *Escherichia coli* (ESBL-EC) and ampicillin-resistant *Enterococcus* spp. (ARE). These bacteria have been selected as examples for clinically relevant resistant bacteria.

Illicit connections in the storm sewers are searched for using Distributed Temperature Sensing (DTS, see e.g. Nienhuis et al., 2013). DTS uses fibre-optic cables that measure in-sewer temperatures along the entire length of the storm sewer (14 km in total). The method yields individual readings for each cables section of 12,5 cm and for every 30 seconds. Locations with temperature anomalies (e.g. sudden temperature increases due to the discharge of warm wastewater) are generally associated with illicit connections.

## 3 RESULTS AND DISCUSSION

Random sampling of surface water and water inside the storm sewer in the Regenboogbuurt was done December 12<sup>th</sup>, 2017. In total 17 samples were collected, of which 5 samples from the western central pond in the area and 12 samples from storm sewer manholes randomly distributed over the area, see Figure 2 (left). In total, ESBL-EC was found in 5 samples (29%) and ARE in 14 samples (82%), see Figure 2 (right).

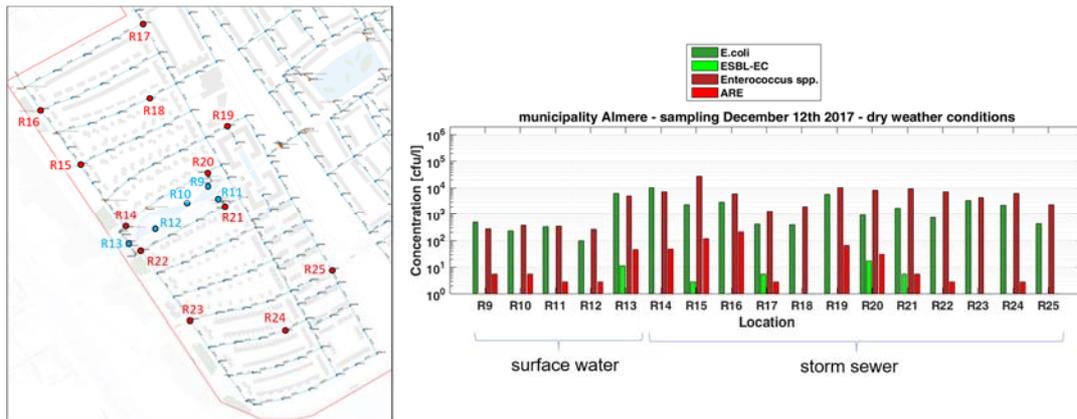


Figure 2. Sampling locations (left) and sampling results (right) for random sampling of *E. coli*, ESBL-EC, *Enterococcus* spp. and ARE in surface water (blue locations) and storm sewer (red locations).

The search for illicit connections resulted in 32 locations with an illicit connection, ranging from a misconnected washing machine to a wrongly connected house connection. Figure 3 (left) shows the illicit connections on the map. Targeted sampling in manholes around illicit connections was done May 22<sup>nd</sup>, 2018. In total 20 samples were collected. In 80% of the samples (16 out of 20) ESBL-EC was found, with concentrations varying between 10<sup>0</sup> and 10<sup>3</sup> cfu/l, see Figure 3 (right).

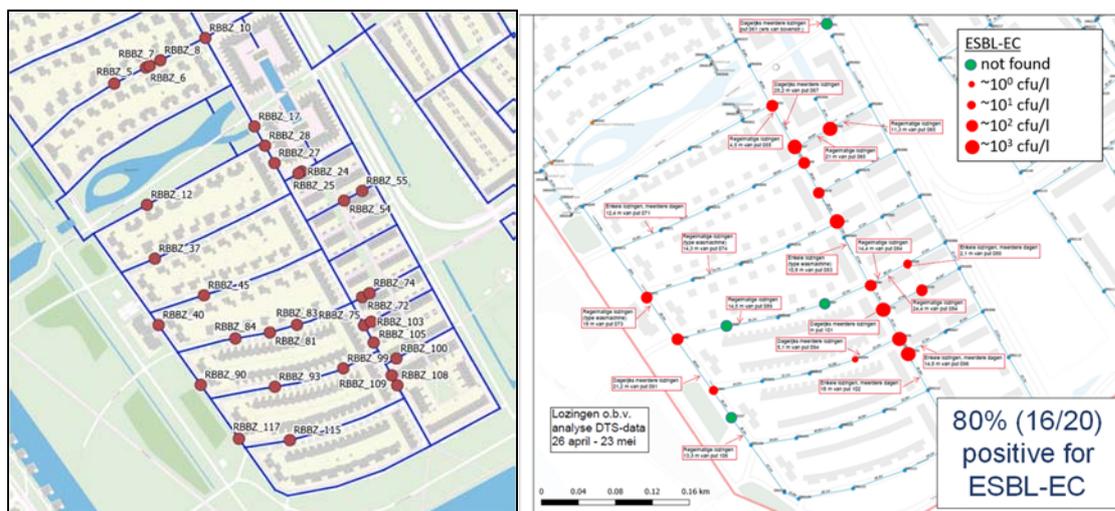


Figure 3. Illicit connections identified (left) and results (right) for sampling of *E. coli*, ESBL-EC in the storm sewer

## 4 CONCLUSIONS - OUTLOOK

The first results of the project show that antibiotic resistant bacteria can be found in storm sewers that are subject to illicit connections. Final results will show whether (or not) the storm sewer will be free of these bacteria after removal of illicit connections.

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