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A comparative study between low- and high-tech methods for the detection and mitigation of illicit connections in stormwater systems

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ABSTRACT

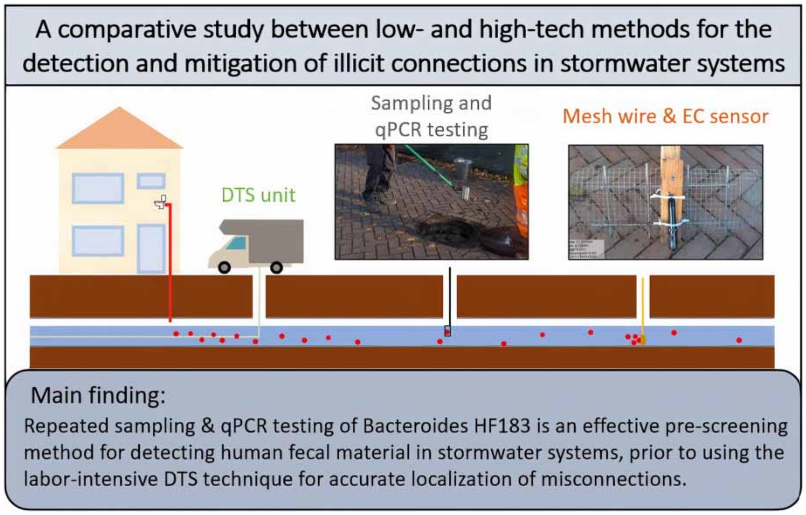
Illicit connections of wastewater to stormwater systems are the main drawback of separate sewer systems, as they lead to a direct discharge of untreated wastewater to the aquatic environment. Consequently, several inspection methods have been developed for detecting illicit connections. This study simultaneously applied several low- and high-tech methods for the detection of illicit connections in the same catchment (De Heuvel, the Netherlands). The methods included mesh wire screens for capturing coarse contamination, measurements of electroconductivity and temperature, sampling and quantification of *Escherichia* coli and extended-spectrum ß-lactamase-producing *E. coli* (ESBL-EC), DNA analysis via quantitative polymerase chain reaction for human-, dog-, and bird-specific fecal indicators, and distributed temperature sensing. Significant illicit connections could be identified using all methods. Nonetheless, hydraulic conditions and, predominantly, the sewage volume determine whether a misconnection can be detected by especially the low-tech methods. Using these results, the identified misconnections were repaired and biological and DNA analyses were repeated. Our results demonstrate that there were no changes in *E. coli* or ESBL-EC before and after mitigation, suggesting that these common markers of fecal contamination are not specific enough to evaluate the performance of mitigation efforts. However, a marked decrease in human wastewater markers (HF183) was observed.

Key words: *Bacteroides*, conductivity, DTS, *E. coli*, ESBL-EC, illicit connections

HIGHLIGHTS

- Wire screens and conductivity are not suited to detect illicit connections at outlets of storm sewers with large water volumes upstream.
- Distributed temperature sensing (DTS) is capable of identifying all sorts of misconnections like household appliances.
- HF183 *Bacteroides* are very effective in determining the presence of human fecal material in storm sewers.
- Repeated sampling and quantitative polymerase chain reaction testing could be a prescreening method before labor-intensive DTS.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Separate sewer systems demonstrate certain advantages over combined systems, including smaller water volumes to the wastewater treatment plant and the absence of combined sewer overflows that lead to the discharge of diluted untreated wastewater to the surrounding receiving water bodies when the system storage is exceeded during rainfall events. However, a common defect observed in separate systems is the existence of illicit connections (Shi *et al.* 2022). Illicit connections pose a threat to the aquatic environment due to the discharge of untreated sewage containing nutrients, pathogens, gross solids, organic micropollutants, and microplastics. Consequently, the detection and localization of illicit connections have often been a subject of research.

Several methods have been proposed for the detection of illicit connections (Panasiuk *et al.* 2015). Some rely on detecting biochemical and/or physical substances in the sewage discharged into the stormwater system. Examples are the quantification of *Escherichia coli* (Irvine *et al.* 2011) or caffeine (Sauvé *et al.* 2012), and the measurement of temperature or electroconductivity (EC) (De Groot *et al.* 2014; Shi *et al.* 2022). Other techniques rely on determining the presence of a physical connection of a sewage discharge point to the stormwater system. These techniques include, among others, smoke tests (Tuomari & Thompson 2004), tracer tests (Pitt *et al.* 2000), visual inspections at storm outlets during dry weather (Irvine *et al.* 2011), infrared camera scanning (Lepot *et al.* 2017), and fiber-optic distributed temperature sensing (DTS) measurements (Hoes *et al.* 2009). Infrared cameras and DTS also provide a spatial resolution within the system allowing for the direct localization of illicit connections under certain conditions.

Each of the described methods is based on a different technological background, yet all methods suffer from specific limitations. For instance, Nienhuis *et al.* (2013) observed a series of limitations regarding DTS measurements, including reduced measurement sensitivity in submerged storm sewers and dependency on the volume of discharged wastewater in case of long household connections. Visual techniques, such as the infrared camera, require that the inspection coincides with the discharge via the illicit connection, while tracer testing requires the cooperation of residents with potential household illicit connections. Furthermore, EC and temperature sensors are generally placed only at a limited number of locations in the system. Similarly, the detection of fecal indicators does not provide any specific information about the discharge point, while more advanced analyses (i.e. DNA) are usually required to distinguish between different sources of fecal contamination (Hu *et al.* 2018; Hachad *et al.* 2022). As a result, every method has its own cost-efficiency relationship.

The objective of the current study is to compare the performance of different methods for detecting illicit connections. For this purpose, a range of low- and high-tech techniques have been applied simultaneously, under identical conditions, in one catchment area in Breda, the Netherlands. The applied methods included the installation and inspection of mesh wire screens, installation and logging of conductivity and temperature sensors, microbiological and DNA analysis of water samples from the studied system, and monitoring with DTS cables. The results from DTS measurements served as a reference in this study since DTS was the most advanced applied method. Suspected illicit connections were validated by a field study during which household appliances were flushed and checked. A unique aspect of this study is that the identified illicit connections were actually repaired, and monitoring was undertaken both before and after rectification works. Our results offer the opportunity to conclude the overall performance of the applied techniques in a stormwater system with challenging hydraulic characteristics and to provide insights on possible technique combinations for achieving higher efficiency with lower effort.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in the stormwater system of catchment De Heuvel in the municipality of Breda, the Netherlands. The catchment is a residential area of 15 ha with storm sewers discharging to a local pond in the southern part of the catchment (Figure 1). The stormwater system has a length of approximately 5.5 km and consists of several parts. The central section (4.5 km) drains under gravity to one of the nine outlets of the central pond. The outlets are fitted with nonreturn valves to prevent inflow from the pond into the system. The southwest part of the system consists of an infiltration transport sewer (0.5 km) with an internal overflow to the central section of the system and an external outfall to the receiving pond. Furthermore, four 'islands' in the area each have their own stormwater system (in total 0.5 km) and outlets; these sewers are not connected to the municipal system and were not included in the study.

The receiving water body consists of a central pond that is hydraulically isolated. Due to its small volume, it is sensitive to contamination from illicit connections. During the visual inspection of manholes before measurements (Breedijk 2017), fecal material (toilet paper, brown solids, grease) was observed in 3 out of 13 inspected manholes, presumably as a result of illicit connections to the system. Further, the foul sewer system was not studied in this research, as the downstream pumping station did not show significant volumes of stormwater during storm events. Therefore, this catchment area was considered suitable for comparing methods for the detection of illicit connections.

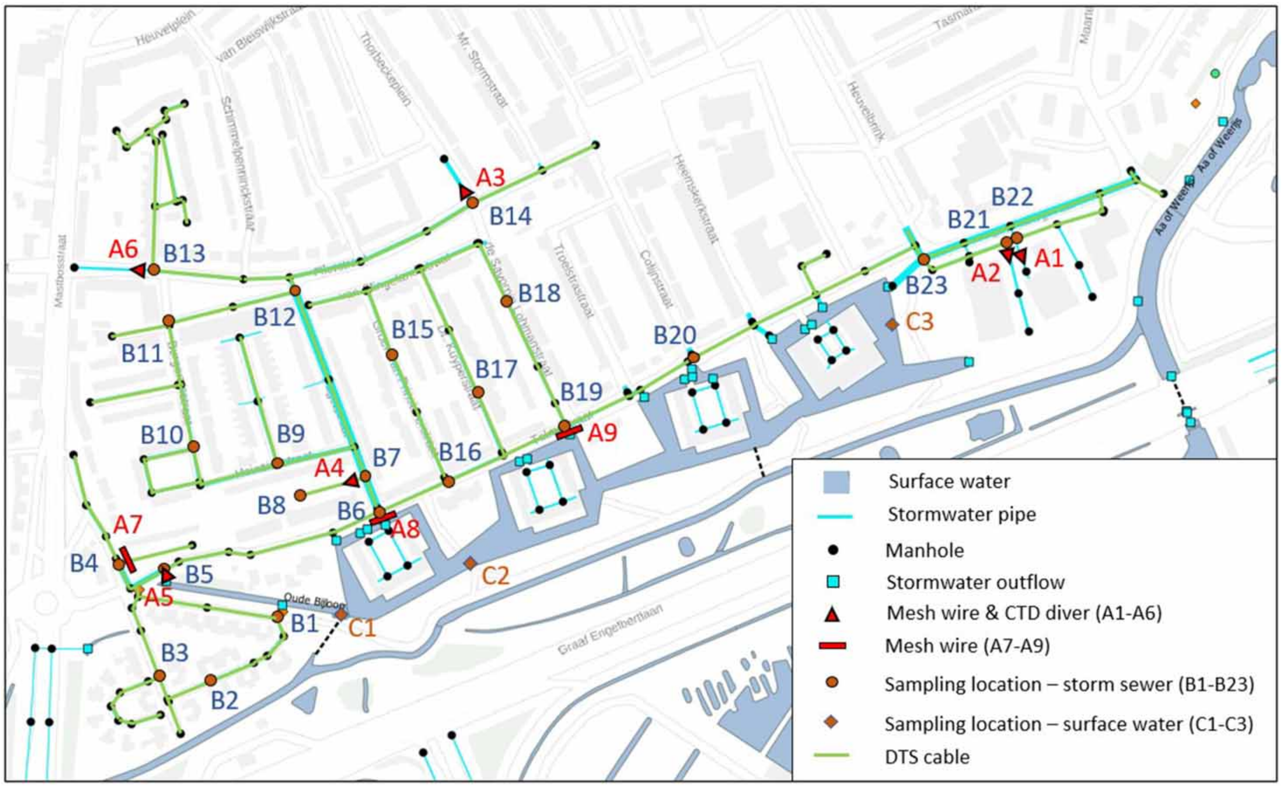


Figure 1 | Catchment area De Heuvel with type and locations of the applied methods/techniques.

2.2. Applied cross connection detection techniques

2.2.1. Mesh wire screens

Mesh wire screens in the stormwater system function as a filter of coarse pollution (e.g. toilet paper, sanitary refuse). Regular inspection of the wire screens for residual solids allows estimation of whether an upstream illicit connection is present. Nine wire screens were installed in the catchment (Figure 1). Five wire screens (A1, A2, A3, A6, and A7) were placed in relatively small upstream pipe sections where no DTS cable was installed. In addition, four wire screens were installed at or near downstream outlets (A4, A5, A8, and A9). The screens were fixated with a wooden beam in a manhole at the invert of the inspected pipe. Screens were made of a 1×1 cm mesh grid and were approximately 40 cm wide and 15 cm high (Figure 3). Their placement and dimensions were such that any dry weather flow would pass through the screen, while stormwater could pass over the screen even in case of extreme pollution of the screens.

2.2.2. Conductivity sensors

Six conductivity sensors (CTD divers®, van Essen Instruments) were utilized, measuring high-frequency conductivity and temperature at various locations in the stormwater system (Figure 1). The idea behind the use of CTD divers in the search for illicit connections is that, due to a different composition, wastewater discharges may be observed as a temporary increase in conductivity and/or temperature. The CTD divers were attached to (a selection of) the same setups as used for the wire screens, hence measuring at the same locations (A1–A6).

2.2.3. Fecal indicators and microbial source tracking methods

Sampling and microbiological analysis of water samples from the stormwater system and from the receiving surface water were conducted to explore the potential relationship between illicit connections and fecal contamination in the system. First, the research focused on the quantification of *E. coli*, a bacterial fecal indicator, and extended-spectrum β-lactamase-producing *E. coli* (ESBL-EC), an antibiotic-resistant variant of *E. coli* that has a prevalence of ~5% in the Dutch population (Van den Bunt *et al.* 2019). Grab samples were filtered and analyzed via selective cultivation on TBX (for *E. coli*) and ChromID ESBL agar (Biomerieux, for ESBL-EC), as described in the study by Blaak *et al.* (2021). For *E. coli*, four to eight different volumes were filtered per sample (ranging between 0.1 and 100 mL), and for ESBL-EC, four volumes (each of 100 mL) were filtered.

Microbiological fecal indicators such as *E. coli* are not specific to human fecal sources entering the sewer system via illicit connections, but can also be of animal origin due to dog and/or bird feces entering the system via stormwater runoff, or animals that live in and around the sewers (e.g. rats). Also, ESBL-EC can originate not only from humans (Van den Bunt *et al.* 2019) but also from dogs (Van den Bunt *et al.* 2020) and waterbirds (Veldman *et al.* 2013).

Therefore, additional DNA analysis of water samples from the storm sewer and receiving water was used to determine the origin of fecal contamination in the water. These analyses targeted bacterial species that occur (almost) exclusively and in high concentrations in the intestines of specific groups, such as humans, dogs, birds, pigs, or cattle (Heijnen *et al.* 2014; Kardinaal 2017). *Bacteroides* gene HF183 was used as a human-specific indicator. Relevant indicators were also used for birds (DNA fraction of a bird-specific *Helicobacter*) and dogs (mitochondrial DNA). Three different methods were used for different sets of water samples: (i) the laboratory of KWR (Water Research Institute) via DNA isolation (Qiagen DNeasy PowerBiofilm kit) and quantification with qualitative polymerase chain reaction (qPCR; for primer sequences see Table S5 in the Supplementary Materials), (ii) 'in situ' by using a small, portable qPCR unit from BioMeme (Franklin™ Real-Time PCR Thermocycler, M1 sample preparation kit, Bacteroides Go-P/P kits plus associated LyoDNA + IPC Go-Strips), and (iii) 'in situ' by using a mobile Orvion Udetect® qPCR unit (hydrobag filtration unit U20027, isolation kit U20030, Target Tube *Bacteroides dorei*).

2.2.4. DTS

The DTS monitoring setup consisted of one central measuring unit with multiple fiber-optic cables in the inspected sewer system. A 3-month monitoring period yielded temperature data every 12.5 cm along the cables at a time interval of 30 s. Analysis of the obtained data was conducted via earlier developed automated data analysis algorithms (Vosse *et al.* 2013). Locations with temperature variations (i.e. a local sudden temperature increase) during dry weather were associated with illicit connections. Based on the observed patterns, the suspected illicit connection was categorized according to the categories presented in Table 1. Category 1 is the most severe illicit connection (in terms of the amount of wastewater discharged to the

Table 1 | Classification of illicit connections

Category	Description
1	Connection between public foul and storm sewers. All sewage of multiple households/enterprises discharged to the storm water system.
2	Single house connection of foul water connected to storm water sewer. All sewage of individual household/enterprise discharged to the storm water system.
3	Falsely connected household appliance only. Example: laundry machine connected to rainwater downpipe. Generally no toilet wastewater.

storm sewer) with multiple households wrongly connected. A pattern with several discharges per day implies a wrongly connected single household (category 2), whereas a limited number of discharges per week would hint at the connection of a single washing machine to the stormwater system (category 3). A field investigation was conducted to verify the DTS findings (mainly using dyes to check toilets and household appliances near an illicit connection).

2.3. Measurement strategy

An empty or nearly empty stormwater system is optimal for the detection of illicit connections since dilution of wastewater in the storm sewer is then limited and water quality and/or temperature variations can be more easily detected. However, even under dry weather conditions, the stormwater system of De Heuvel is completely submerged around the outlets (same water level as in the pond). Further upstream the system becomes gradually empty. To facilitate the detection of illicit connections with DTS and to allow the installation of wire screens and CTD divers, the system was pumped (nearly) empty several times during this study with a mobile pump unit. The water level in the stormwater system was monitored at four locations (B5, B6, B19, and B23 in Figure 1). Precipitation data were obtained from the Hydronet rain radar and a local station of the Royal Netherlands Meteorological Institute (KNMI).

The chronology of the application of the four detection techniques is presented in Table 2. Sampling from the storm sewer and receiving water was executed in three rounds: in June and November 2019 in the original state (illicit connections still present), and in September 2022 after repair of illicit connections in March 2020 (the prolonged delay between repairs and sampling was due to the COVID-19 pandemic). The 23 sampling locations from the storm sewer (B1–B23) and the 3 locations

Table 2 | Chronology applied detection techniques

Period	Action	Sampling locations	Sample analyses
5, 11, 18 June 2019	Water sampling original state #1	B1, B6, B12, B19, B23 C1, C2, C3	*E. coli*, ESBL-EC (RIVM, culture based) DNA (human, dog, and bird, KWR laboratory)
October to December 2019	DTS monitoring (net 26 days of data suitable for data analysis)		
28 October 2019	Installation wire screens and CTD divers		
7 November 2019	First inspection of wire screens		
20 November 2019	Second inspection of wire screens, removal of screens and CTD divers		
25–28 November 2019	Water sampling original state #2	B1–B22 C1, C2, C3	DNA (human, BioMeme qPCR unit *in situ*)
March 2020	Repair of illicit connections		
19–22 September 2022	Water sampling after removing illicit connections	B1–B20 and B23 C1, C2, C3 B1, B6, B12, B19, B23 C1, C2, C3	DNA (human, Orvion Udetect qPCR unit *in situ*) *E. coli*, ESBL-EC (RIVM, culture based)

from the pond (C1–C3) are shown in Figure 1. The DTS monitoring was performed between October and December 2019 yielding a total of 26 days of data fit for data analysis (DTS data collected during storm events are not suitable for analysis). The wire screens and CTD divers were installed on 28 October 2019. The screens were inspected on 7 November and prior to their removal on 20 November 2019.

3. RESULTS

3.1. DTS measurements

Frequent and/or large temperature deviations in the DTS measurements were found at nine locations in the sewer system (Figure 2). These locations were further examined by conducting systematic appliance discharges (e.g. in toilets, bathrooms, washing machines) in the houses around the detected discharge points.

Location DTS 9 proved a large illicit connection with all wastewater from a three-storey building (three households) misconnected to the storm sewer. This illicit connection was repaired in March 2020. At location DTS 5, a visual inspection of the manhole revealed that wastewater (including fecal material) was present in the stormwater system, but the exact source could not be determined during the site visit. As a result, this illicit connection was not (yet) repaired by September 2022.

The other seven locations proved to be misconnections in the in-house plumbing, resulting in nonfecal discharges (e.g. washing machines) to the stormwater system. Locations DTS 7 and DTS 8 were repaired, and the other locations were not due to the practical (in)feasibility of repair (as judged by the municipality).

3.2. Mesh wire screens

Figure 3 shows a selection of results of the wire screens. No signs of fecal contamination (i.e. no toilet paper or fecal matter) were found on the majority of the inspected screens, although (remnants of) leaves were found at some locations. Only the screen at location A4 (downstream of DTS 9 where sewage was detected) showed clear traces of greasy contamination and a distinct sewage smell during the inspections.

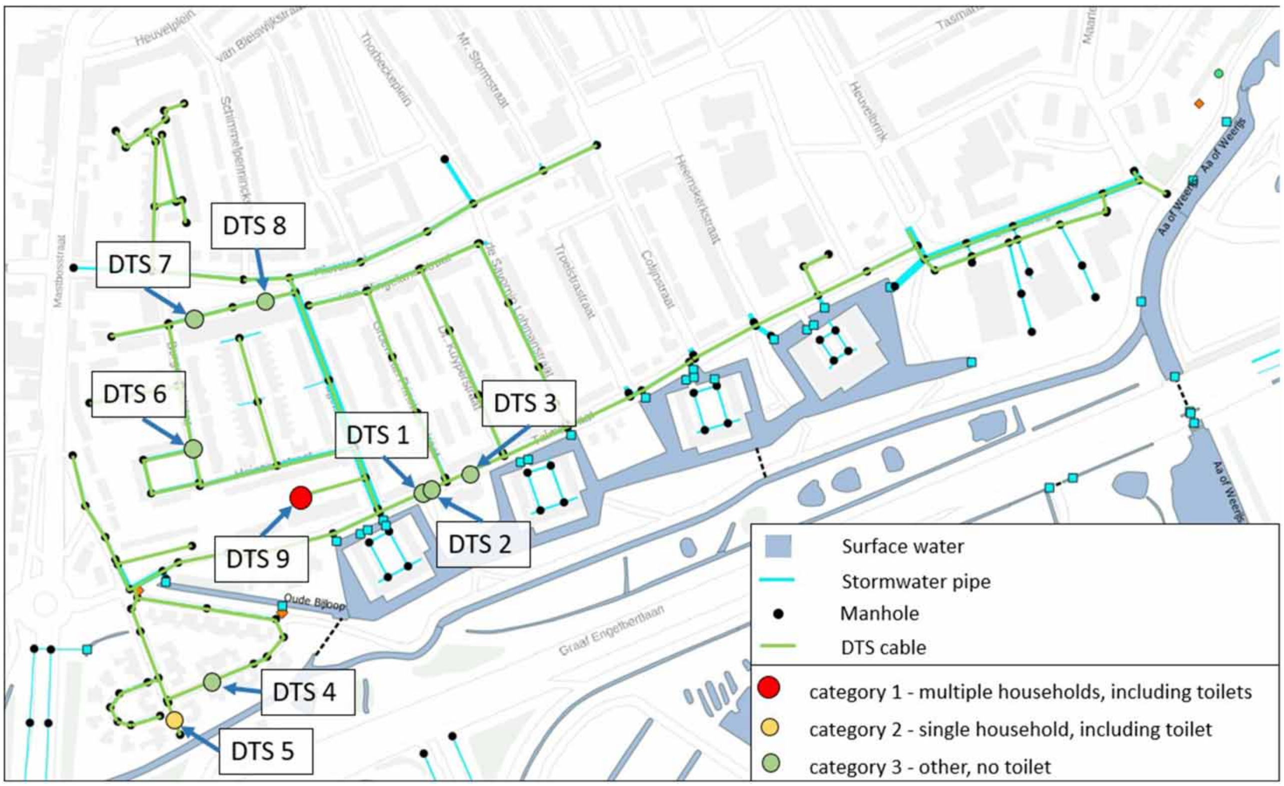


Figure 2 | Results of DTS monitoring with categorization of illicit connections.

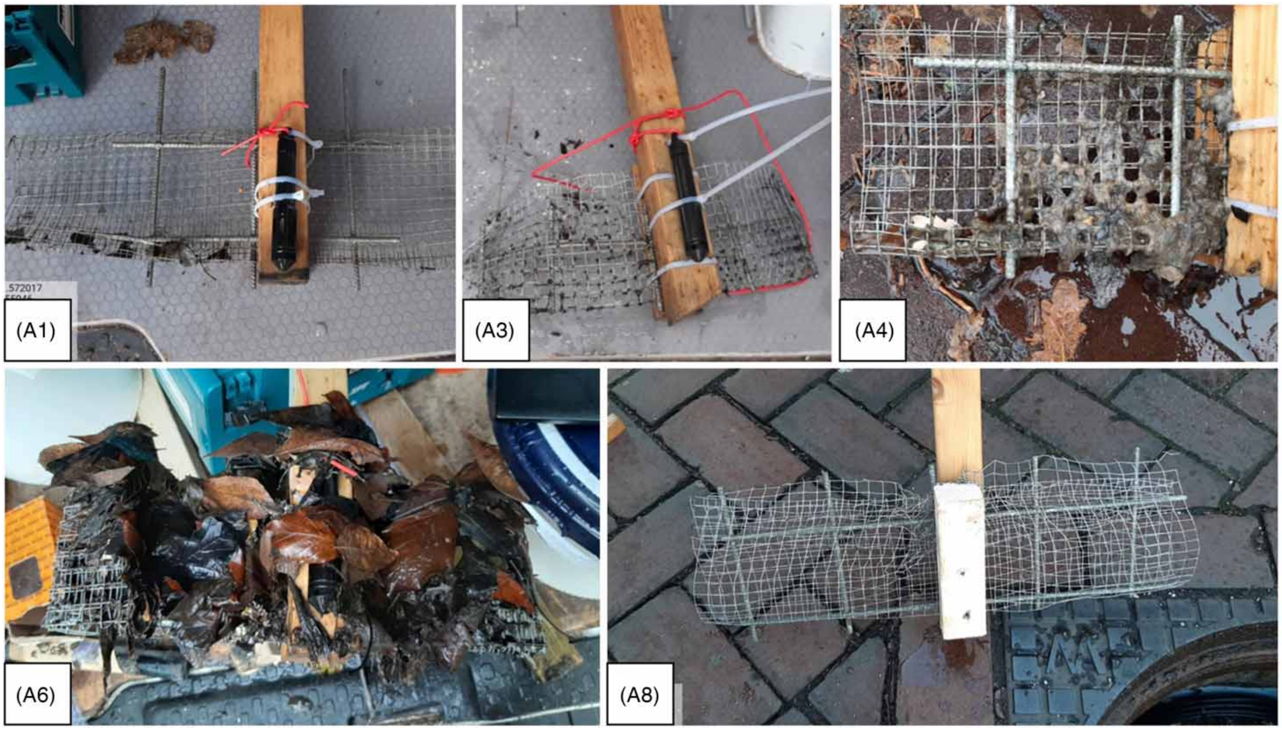


Figure 3 | Mesh wire screens during inspection in November 2019.

3.3. Conductivity and temperature measurements

For installation of the CTD divers on 28 October 2019, the storm sewer was emptied using a mobile pump installation. As a result, during the first days of installation, monitoring results were absent (conductivity) or nonrepresentative (measuring air temperatures). After a storm event on 1 November, the water level in the storm sewer returned to normal, leaving the divers submerged in water levels between 25 and 110 cm, see Table 3.

The same pattern was observed at almost all locations, with constant temperature and conductivity values during dry weather and more variability in both parameters during storm events (results are presented in Figures S1–S5 in Supplementary Material). The inflow of stormwater runoff is often colder and has a more variable composition than the water already in the storm sewer. The only exception to this finding was again at location A4 where variations in electrical conductivity were observed during dry weather periods (Figure 4). These variations occurred at high frequency with several peaks per day and higher absolute values of 400–500 μS/cm, whereas at other locations, the maximum values were around 200 μS/cm.

3.4. Fecal indicators

Figure 5 shows an overview of the analysis results for *E. coli* and ESBL-EC for samples collected before and after the repair of illicit connections. *E. coli* was detected in all samples from the stormwater system as well as from the pond. Removal of illicit

Table 3 | Hydraulic conditions near CTD divers and data observations

Location	Pipe diameter (mm)	Water level (cm) at CTD	Results – observations
A1	250	~30	Variations only during precipitation
A2	315	~30	Variations only during precipitation
A3	500	~90	Variations only during precipitation
A4	250	~35	Variations during dry weather as well as large absolute values (Figure 4)
A5	700	~110	Variations only during precipitation
A6	315	~25	Variations only during precipitation

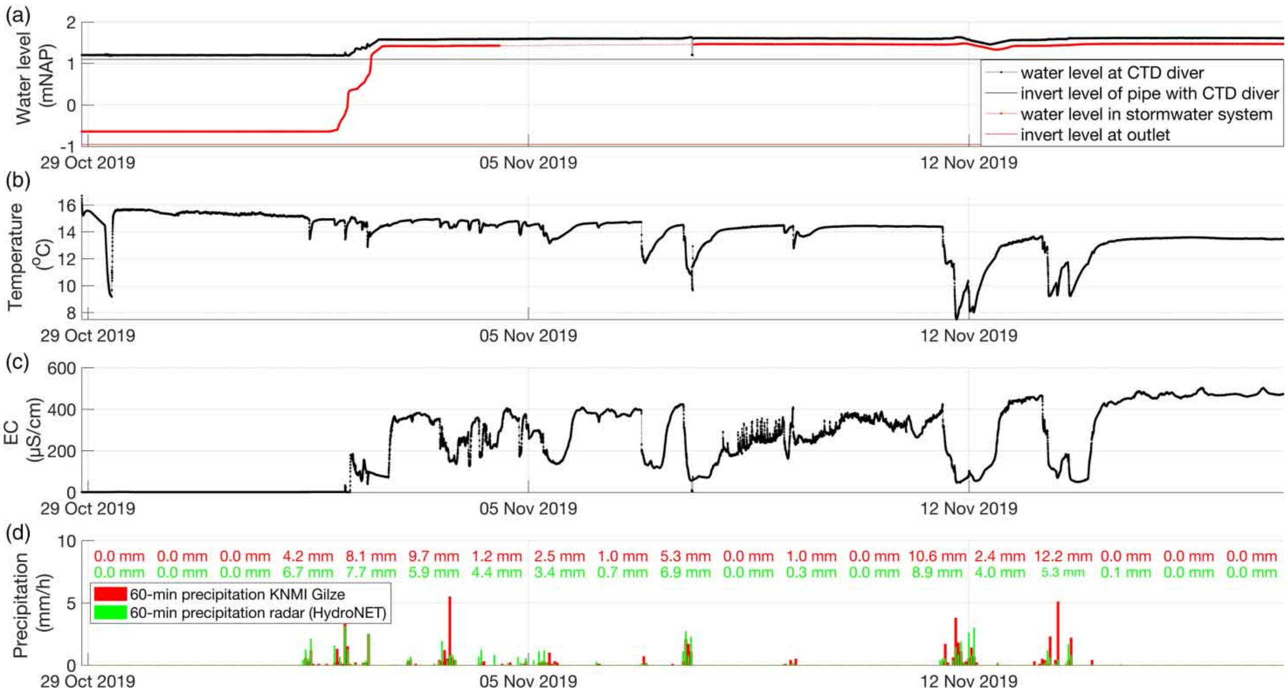


Figure 4 | CTD diver® data at location A4: (a) water levels, (b) temperature, (c) electrical conductivity, and (d) precipitation.

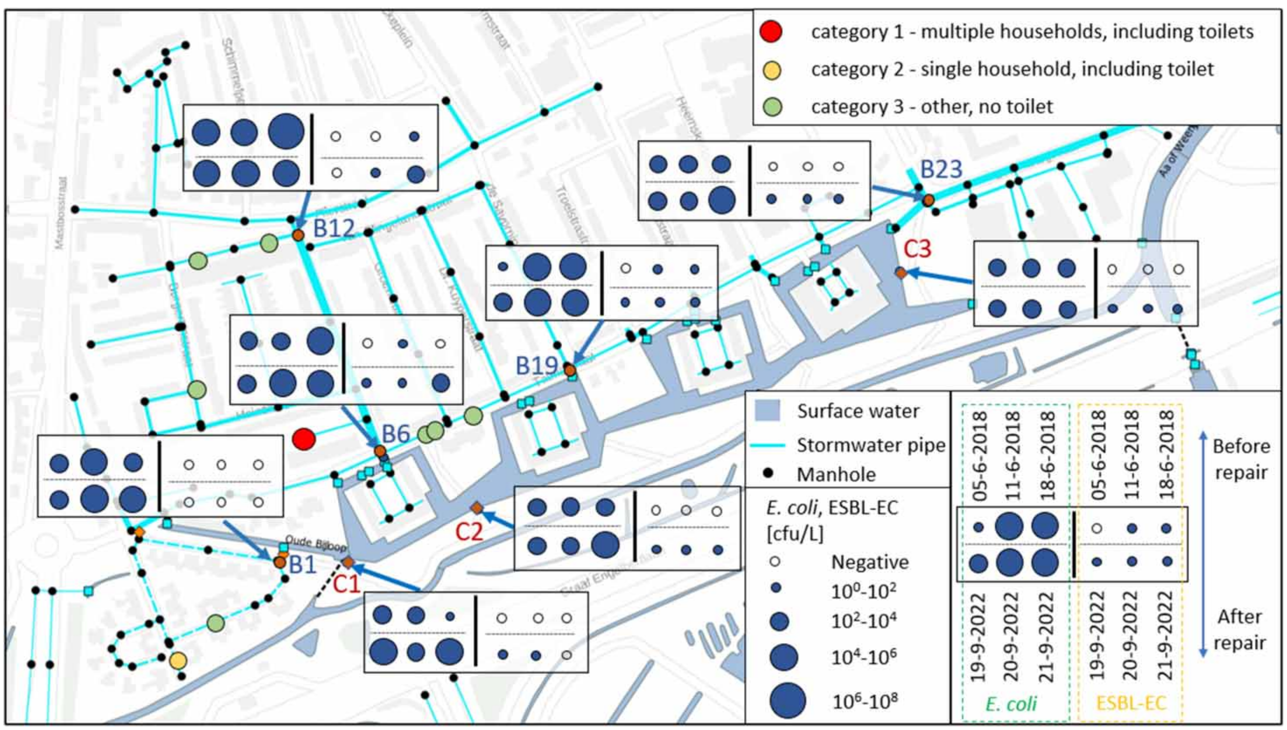


Figure 5 | *E. coli* (per location left-hand side) and ESBL-EC (right-hand side) concentrations in June 2019 (before repair of illicit connections, per location upper row) and in September 2022 (after repair, bottom row). Results are categorized according to their (log)value. Analysis results are given in Table S1 in Supplementary Material. The locations of the illicit connections found with DTS are also indicated in the figure.

connections did not result in the expected reduction of *E. coli* concentrations. In contrast, the average storm sewer concentrations were about three times higher in 2022 compared to that in 2019 (geometric mean 5.7×10^3 vs. 1.9×10^4 cfu/L, respectively). Especially the consistently high concentrations at location B6 (two manholes downstream of the removed category 1 illicit connection) were unexpected.

For ESBL-EC, 23 of a total of 48 samples (48%) were found positive. Again, the removal of illicit connections did not lead to lower concentrations, but rather the contrary: the proportion of positive samples increased from 17% (2019) to 79% (2022). The share of ESBL-EC in the total amount of *E. coli* was approximately 0.001% for 1 sample, 0.01–0.1% for 9 samples, and 0.1–1% for 13 samples.

3.5. Source tracking markers

Figure 6 shows the results of DNA analysis for human markers. The human-specific DNA marker was found in 60 of a total of 214 samples (28%). There is a large difference between the samples before repair of illicit connections (44% positive) and after repair (8% positive). Moreover, five of the eight positive samples from September 2022 were collected at locations B2 and B3 near the unresolved illicit connection at location DTS 5 (yellow dot). The results at locations B8, B7, and B6 show a strong effect associated with the repair of the illicit connection at location DTS 9 (large red dot): consistently positive results before repair and consistently negative results after repair.

In June 2019, dog DNA marker was found in 6 of the 15 samples (40%), all collected from the stormwater system. Bird-specific DNA marker was found only in one sample from the pond (location C2).

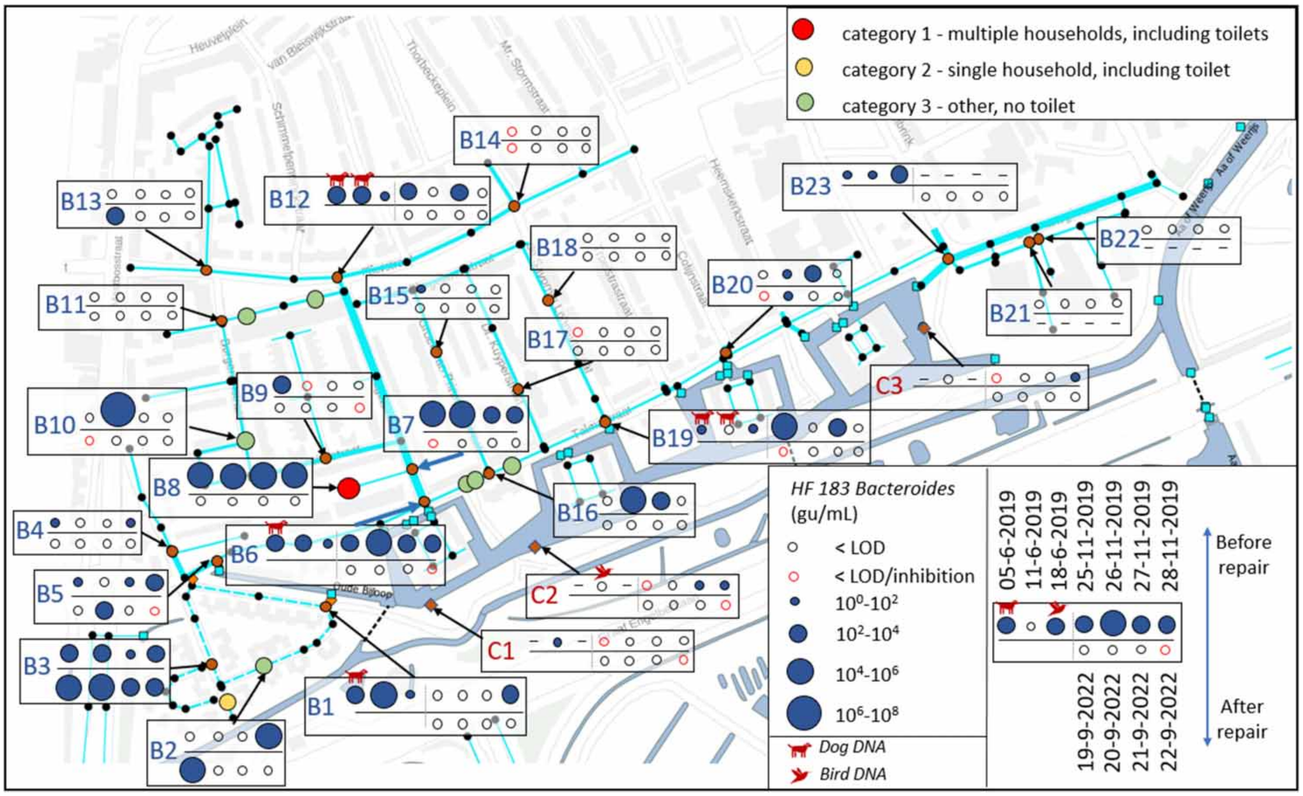


Figure 6 | Human-specific DNA results before (June and November 2019, per location upper row) and after (September 2022, bottom row) repair of illicit connections. Dog and/or bird-specific DNA marker was analyzed only for samples collected in June 2019; a positive sample for these targets is indicated with a red symbol. Detailed analysis results are given in Tables S2–S4 in Supplementary Material. While the absolute concentrations might not be directly comparable due to the different methods used, the orders of magnitude of the results as shown in this figure are considered valid and as such allow comparison.

4. DISCUSSION

4.1. Basic inspection techniques: mesh wire screens and conductivity measurements

The mesh wire screens are limited to illicit connections that generate coarse contamination. As a result, discharges from a washing machine are not detectable. The wire screen at location A4 was installed one manhole downstream of a category 1 illicit connection (DTS 9) that discharged all wastewater from multiple households to the storm sewer. The contamination found on this screen was therefore in line with the presence of a misconnection. At the screen, at location A8 (situated approximately 30 m further downstream at the outlet to the pond), however, no contamination was observed. This is possibly the result of disintegration and dilution of pollutants in the relatively large in-sewer volume between the two screens (submerged 800 mm pipe, approximately 40 m^3). Another possibility is that the upstream A4 screen held back most of the contamination. At the other screens at outlets to the pond (A5 and A9), no contaminants were observed, which is in line with only category 3 illicit connections upstream of these outlets.

Hence, in this study, installing wire screens only at stormwater sewer outlets did not prove to be a suitable method for screening illicit connections. The hydraulic conditions in the studied storm sewer seem to play a critical role here: large in-sewer volumes of water just upstream of the outlets are likely to have prevented any coarse materials 'reaching' the screens. In situations with only small in-sewer volumes upstream of the screen (approximately 2.5 m^3 in the case of screen A4), the screen does indicate the presence of an upstream illicit connection. For other screens installed under similar conditions and that contained no pollution (A1, A2, A3, A6, and A7), the conclusion seems justified that no upstream illicit connection is present in the storm sewer.

For the conductivity and temperature measurements, the results were comparable to those for the mesh wire screens. The category 1 illicit connection at location DTS 9 was visible at location A4 via the EC signal (high-frequency variations and high absolute values during dry weather situations that fit the discharge of wastewater), but was not visible in the EC measurements at the downstream outlet A8. Again, the difference in hydraulic conditions is probably the explanation for this difference: the large upstream in-sewer water volume may attenuate the variations in EC signal to the point that these can no longer be distinguished in the data. EC monitoring at outlets proved insufficient in this sewer system to detect illicit connections. Similar to the wire screens, the absence of dry weather variations in the EC signals at locations without large upstream in-sewer volumes (A1, A2, and A6) confirms the earlier conclusion that no illicit connections are present in these pipes.

The sensitivity of CTD divers could be increased by (temporarily) lowering the water level in the stormwater systems via pumping. For sufficient water depth at the monitoring locations, an obstacle (e.g. a sandbag, see Shi *et al.* 2022) is required. The use of CTD divers in 'empty' stormwater systems has been studied earlier (De Groot *et al.* 2014). Their results showed that a CTD diver can detect an illicit connection up to several hundred meters further downstream on the conditions of sufficient pipe slope and no dominant infiltration of groundwater. Therefore, there is a range of mainly hydraulic parameters that affect the detection accuracy of CTD divers. Accordingly, localization of the misconnections seems impossible, partly due to the observed low accuracy in most cases and partly due to the theoretically possible application points at manholes or outlets. This is a noted issue also for the mesh wire screens.

4.2. Water sampling and microbiological analyses

Analysis of *E. coli* showed quantifiable concentrations in every sample from both the stormwater system and the surface water, implying a certain degree of fecal contamination in the entire system. Although relatively high concentrations were found near the category 1 illicit connection before repair, the repair itself did not lead to a decrease in observed concentration values. Apparently, at the time of sampling, the water in the storm sewer contained *E. coli* also from other sources (such as dog and bird feces). As a result, *E. coli* proved to be ineffective as an indicator for the presence or mitigation of illicit connections in the studied storm sewer.

ESBL-EC has been tested as a fecal indicator in recent studies (Jørgensen *et al.* 2017; Asaduzzaman *et al.* 2022). In the current study, ESBL-EC was found in 48% of samples, in both the storm sewer and the pond. While it appears counterintuitive that the percentage of ESBL-EC positive samples increased after repair, this might be linked to the higher concentrations of total *E. coli* in the 2022 samples, which also increases the likelihood of ESBL-EC concentrations (if present) exceeding the detection limit at a given ESBL-EC-to-*E. coli* proportion. The observed proportion of ESBL-EC among *E. coli* in storm sewers and surface water varied between approximately 0.001 and 1%, which is relatively low compared to the typical share

(0.5–1%) in wastewater of human origin (Jørgensen *et al.* 2017; Schmitt *et al.* 2017). Intriguingly, the large share of samples that were positive for ESBL-EC after repair contrasted with the DNA analysis results, which confirm that the (central part of the) storm sewer was nearly 'free' of human-specific DNA (and hence human feces). This combination of results suggests that the observed ESBL-EC in the sewer probably originated from non-human sources. A potential source could be animal feces that ended up in the storm sewer via stormwater runoff, such as dog feces. Dogs are known carriers of ESBL-EC with a substantial prevalence of carriage (circa 11%, Van den Bunt *et al.* 2020). DNA of dogs was found in samples from the storm sewer before the removal of illicit connections in June 2019, but samples from September 2022 (after repairs) were not tested for dog DNA. As for *E. coli*, thus, the possibility of other contributing sources makes ESBL-EC unfit to use as an indicator for the presence (or localization) of illicit connections in storm sewers.

Analysis results suggest that the human-specific DNA marker *Bacteroides* (HF183) is a good indicator for the presence (and localization) of an illicit connection in a storm sewer, which is in line with the literature (Ahmed *et al.* 2008). The results at location B8 (and B7 and B6 in the downstream direction) show a strong effect associated with the repair of the illicit connection at location DTS 9: consistently positive results before repair and consistently negative results after repair. Results at other locations, however, do show some inconsistencies. For instance, the sample collected on 26 November 2019 at location B10 demonstrated a high concentration value (2.2×0^7 gene copies/mL), while the samples collected 1 day earlier and later were both negative. As this strong variation seems unlikely at such a short time span and as downstream locations are negative for the same day, it is suspected that the sudden positive result is an anomaly due to an unknown cause. At locations B5, B12, B16, and B19, we observed intermittently positive and negative results. These locations are, however, not directly downstream of a known illicit connection with human fecal material. Possibly, a temporarily altered flow direction in the system related to precipitation may have caused water from the category 1 illicit connection (DTS 9) to move around the stormwater sewer, or the DTS system was unable to detect yet another intermittent source of human pollution.

The presence of this variability in the dataset shows the need for repeated sampling on consecutive days when using a human-specific DNA marker as a tracer for illicit connections. The results suggest that the most contaminating (and hence most important) illicit connections can be identified by consistently positive results. The observed variability can be a product of numerous factors that increase the levels of uncertainty in the results. Repeated sampling and analysis are essential to consider the uncertainties present during the processes of sampling, storage, and analytical measurements (McCarthy *et al.* 2008). Finally, accurate localization of an illicit connection even with the more advanced microbiological analyses is difficult due to the limited sampling locations (i.e. via manholes). Only the DTS technique can be used for more accurate localization of illicit connections due to its dense spatial resolution, although its sensitivity may be hindered due to certain parameters, such as the temperature difference between stormwater in the system and illicit discharge, the water level in the system, and the length of the house connection (Nienhuis *et al.* 2013). Therefore, the use of *Bacteroides* HF183 could be an effective indicator of the existence of an illicit connection to a specific part of the stormwater system, before deploying the more costly and labor-intensive DTS technique for a more accurate localization of the misconnection.

4.3. Impact of precipitation during sampling on DNA analyses

Precipitation has two potentially opposite effects on the concentrations of human-specific DNA markers found in the stormwater system: (i) dilution and (ii) distribution. The inflow of stormwater runoff generally leads to dilution and hence lower concentrations in the sewer. On the other hand, the inflow of stormwater runoff may also cause wastewater from illicit connections to spread more through the storm sewer and hence increase concentrations further away from the illicit connection.

Details on precipitation around the sampling days in June and November 2019 and September 2022 can be found in the Supplementary Material (Figure S6). There is no clear effect in the DNA data related to storm events. Samples taken on dry weather days (18 June and 25, 26 November) do not produce consistently higher or lower concentrations. Conversely, samples taken on days with or just after precipitation (5, 11 June, 27, 28 November, e.g. at locations B3, B6, B7, and B8) do not demonstrate significantly lower concentrations. In this context, the relatively high concentrations of human-specific DNA markers in the storm sewer of up to 10^7 gene copies/mL are of particular importance. Even with a dilution rate of 10 or even 100 after a storm event, the resulting DNA marker concentrations remain well above the detection limit. This is in contrast with other 'classical' parameters (such as temperature, NH_4, conductivity) that are used to trace illicit connections in storm sewers.

The presence of dog feces in the storm sewer is expected to show a much stronger relation with storm events as stormwater runoff is the main transport route from street to sewer. Although only a limited number of samples were analyzed for dog

DNA markers, the results seem to confirm this relationship. All six positive samples for dog DNA marker were obtained on days with or just after precipitation (5 and 11 June). In the samples of 18 June (after two to three dry weather days), no dog DNA marker was found.

5. CONCLUSIONS

Different techniques were utilized to search for illicit connections in the stormwater system (5.5 km length) of the De Heuvel area in the municipality of Breda (the Netherlands). These techniques included DTS, temperature and EC measurements (CTD divers), mesh wire screens, and sampling and analysis for *E. coli*, ESBL-EC, and DNA markers. DTS results were used as a reference since these could provide the location and frequency of wastewater discharges. In total, nine illicit connections were localized in the storm sewer with DTS. Field investigations showed that (1) one apartment block with three households was wrongly connected, (2) a single home was wrongly connected, and (3) at seven locations, mistakes in the indoor plumbing caused laundry machines to discharge to the storm sewer (no toilets).

Mesh wire screens and CTD divers measuring EC and temperature proved to be ineffective to detect these illicit connections unless they were installed very close to the source. The hydraulic conditions in the studied storm sewer probably played a critical role in the accuracy of these techniques: large in-sewer volumes of water just upstream of the outlets likely prevented any coarse materials to 'reach' screens at the outlets and diluted EC and temperature signals.

Extensive sampling and microbiological analysis before and after the repair of (a selection of) illicit connections in the stormwater system showed that the human-specific *Bacteroides* gene HF183 worked well to detect the presence and the mitigation of an illicit connection. By contrast, neither *E. coli* nor ESBL-EC was able to consistently detect the presence or repair of an illicit connection. This key finding has a significant impact on future practice as *E. coli* is commonly used as an indicator for illicit connections, yet our study demonstrates the inability to use this organism to assess the performance of mitigation. After repair, *E. coli* and ESBL-EC were still found in many samples, while human-specific DNA markers were essentially absent in the storm sewer. Animal (e.g. dog) feces entering the sewer via stormwater runoff could be a possible source for the observed *E. coli* and ESBL-EC concentrations. A sampling campaign followed by DNA analysis for human markers can therefore be used to investigate a stormwater system for illicit connections. A precondition is that sampling should take place at a sufficient number of locations and on several consecutive days to prevent incorrect conclusions due to the expected uncertainties and inconsistencies in the results. Finally, the deployment of the DTS technique for the accurate localization of an illicit connection could be limited only to parts of the systems where human-specific DNA markers are consistently detected.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Ahmed, W., Stewart, J., Powell, D. & Gardner, T. 2008 Evaluation of Bacteroides markers for the detection of human faecal pollution. *Letters in Applied Microbiology* **46** (2), 237–242. https://doi.org/10.1111/j.1472-765X.2007.02287.x.

Asaduzzaman, M., Rousham, E., Unicomb, L., Islam, M. R., Amin, M. B., Rahman, M., Hossain, M. I., Mahmud, Z. H., Szegner, M., Wood, P. & Islam, M. A. 2022 Spatiotemporal distribution of antimicrobial resistant organisms in different water environments in urban and rural settings of Bangladesh. *Science of the Total Environment* **831**, 154890. https://doi.org/10.1016/j.scitotenv.2022.154890.

Blaak, H., Kemper, M. A., De Man, H., Van Leuken, J. P. G., Schijven, J. F., Van Passel, M. W. J., Schmitt, H. & De Roda Husman, A. M. 2021 Nationwide surveillance reveals frequent detection of carbapenemase-producing Enterobacterales in Dutch municipal wastewater. *Science of The Total Environment* **776**, 145925. https://doi.org/10.1016/j.scitotenv.2021.145925.

Breedijk, J. 2017 Exploring illicit connections in Breda (in Dutch). Een gebiedsgerichte aanpak van foute rioolaansluitingen in de Gemeente Breda. MSc project TU Delft.

De Groot, A. C., de Haan, C., Klootwijk, M., Schilperoort, R. & Langeveld, J. 2014 'Yes, we scan!': ontwikkeling Quickscan opsporen foutaansluitingen regenwateriolen (in Dutch: quickscan for the detection of illicit connections in storm sewers). *Vakblad Riolering* **21** (2014), 24–26. Available from: http://library.wur.nl/WebQuery/hydrotheek/2066371.

Hachad, M., Lanoue, M., Duy, S. V., Villemur, R., Sauvé, S., Prévost, M. & Dorner, S. 2022 Locating illicit discharges in storm sewers in urban areas using multi-parameter source tracking: field validation of a toolbox composite index to prioritize high risk areas. *Science of the Total Environment* **811**, 152060. https://doi.org/10.1016/j.scitotenv.2021.152060.

Heijnen, L., Learbuch, K., Kardinaal, E., Rotteveel, S., Ruiter, H. & Leenen, I. 2014 Identifying fecal contamination in bathing water with DNA-indicators (Fecale verontreiniging in zwemwater identificeren met DNA-merkers). H_2O online. Available from: https://www.h2owaternetwerk.nl/images/2014/1404/1404-04_DNAdetectieFeces.pdf.

Hoes, O. A. C., Schilperoort, R. P. S., Luxemburg, W. M. J., Clemens, F. H. L. R. & Van De Giesen, N. C. 2009 Locating illicit connections in storm water sewers using fiber-optic distributed temperature sensing. *Water Research* **43** (20), 5187–5197. https://doi.org/10.1016/j.watres.2009.08.020.

Hu, Y. O., Ndegwa, N., Alneberg, J., Johansson, S., Logue, J. B., Huss, M., Käller, M., Lundeberg, J., Fagerberg, J. & Andersson, A. F. 2018 Stationary and portable sequencing-based approaches for tracing wastewater contamination in urban stormwater systems. *Scientific Reports* **8** (1), 1–13. https://doi.org/10.1038/s41598-018-29920-7.

Irvine, K., Rossi, M. C., Vermette, S., Bakert, J. & Kleinfelder, K. 2011 Illicit discharge detection and elimination: low cost options for source identification and trackdown in stormwater systems. *Urban Water Journal* **8** (6), 379–395. https://doi.org/10.1080/1573062X.2011.630095.

Jørgensen, S. B., Søraas, A. V., Arnesen, L. S., Leegaard, T. M., Sundsfjord, A. & Jenum, P. A. 2017 A comparison of extended spectrum *β*-lactamase producing *Escherichia coli* from clinical, recreational water and wastewater samples associated in time and location. *PloS One* **12** (10), e0186576. https://doi.org/10.1371/journal.pone.0186576.

Kardinaal, E. 2017 Source tracing of fecal contamination in bathing water 2017 (Bronopsporen fecale verontreiniging in zwemwater 2017). KWR 2017.077. Available from: https://edepot.wur.nl/454731.

Lepot, M., Makris, K. F. & Clemens, F. H. 2017 Detection and quantification of lateral, illicit connections and infiltration in sewers with infra-red camera: conclusions after a wide experimental plan. *Water Research* **122**, 678–691. https://doi.org/10.1016/j.watres.2017.06.030.

McCarthy, D. T., Deletic, A., Mitchell, V. G., Fletcher, T. D. & Diaper, C. 2008 Uncertainties in stormwater *E. coli* levels. *Water Research* **42** (6–7), 1812–1824. https://doi.org/10.1016/j.watres.2007.11.009.

Nienhuis, J., De Haan, C., Langeveld, J., Klootwijk, M. & Clemens, F. 2013 Assessment of detection limits of fiber-optic distributed temperature sensing for detection of illicit connections. *Water Science and Technology* **67** (12), 2712–2718. https://doi.org/10.2166/wst.2013.176.

Panasiuk, O., Hedström, A., Marsalek, J., Ashley, R. M. & Viklander, M. 2015 Contamination of stormwater by wastewater: a review of detection methods. *Journal of Environmental Management* **152**, 241–250. https://doi.org/10.1016/j.jenvman.2015.01.050.

Pitt, R., Labor, M., Harper, J., Nix, C. & Barbe, D. 2000 Potential new tools for the use of tracers to indicate sources of contaminants to storm drainage systems. In: *U. S. Environmental Protection Agency, National Conference on Tools for Urban Water Resource Management and Protection: Proceedings*, pp. 97–109.

Sauvé, S., Aboulfadl, K., Dorner, S., Payment, P., Deschamps, G. & Prévost, M. 2012 Fecal coliforms, caffeine and carbamazepine in stormwater collection systems in a large urban area. *Chemosphere* **86** (2), 118–123. https://doi.org/10.1016/j.chemosphere.2011.09.033.

Schmitt, H., Blaak, H., Kemper, M., van Passel, M. W., Hierink, F., van Leuken, J., de Roda-Husman, A. M., van der Grinten, E., Rutgers, M., Schijven, J., de Man, H., Hoeksma, P. & Zuidema, T. 2017 Sources of antibiotic-resistant bacteria in the environment and possible measures (Bronnen van antibioticaresistente bacteriën in het milieu en mogelijke maatregelen). RIVM Rapport 2017-0085. https://doi.org/10.21945/RIVM-2017-0058.

Shi, B., Catsamas, S., Deletic, B., Wang, M., Bach, P. M., Lintern, A., Deletic, A. & McCarthy, D. T. 2022 Illicit discharge detection in stormwater drains using an Arduino-based low-cost sensor network. *Water Science and Technology* **85** (5), 1372–1383. https://doi.org/10.2166/wst.2022.034.

Tuomari, D. C. & Thompson, S. 2004 'Sherlocks of stormwater' effective investigation techniques for illicit connection and discharge detection. In: *Proceedings of the Water Environment Federation: WEFTEC 2004, New Orleans*. Water Environment Federation, pp. 1252–1259.

Van den Bunt, G., van Pelt, W., Hidalgo, L., Scharringa, J., de Greeff, S. C., Schürch, A. C., Mughini-Gras, L., Bonten, M. J. & Fluit, A. C. 2019 Prevalence, risk factors and genetic characterisation of extended-spectrum beta-lactamase and carbapenemase-producing Enterobacteriaceae (ESBL-E and CPE): a community-based cross-sectional study, The Netherlands, 2014 to 2016. *Eurosurveillance* **24** (41), 1800594. https://doi.org/10.2807/1560-7917.ES.2019.24.41.1800594.

Van den Bunt, G., Fluit, A. C., Spaninks, M. P., Timmerman, A. J., Geurts, Y., Kant, A., Scharringa, J., Mevius, D., Wagenaar, J. A., Bonten, M. J., van Pelt, W. & Hordijk, J. 2020 Faecal carriage, risk factors, acquisition and persistence of ESBL-producing Enterobacteriaceae in dogs and cats and co-carriage with humans belonging to the same household. *Journal of Antimicrobial Chemotherapy* **75** (2), 342–350. https://doi.org/10.1093/jac/dkz462.

Veldman, K., Van Tulden, P., Kant, A., Testerink, J. & Mevius, D. 2013 Characteristics of cefotaxime-resistant *Escherichia coli* from wild birds in The Netherlands. *Applied and Environmental Microbiology* **79** (24), 7556–7561. https://doi.org/10.1128/AEM.01880-13.

Vosse, M., Schilperoort, R., de Haan, C., Nienhuis, J., Tirion, M. & Langeveld, J. 2013 Processing of DTS monitoring results: automated detection of illicit connections. *Water Practice and Technology* **8** (3–4), 375–381. https://doi.org/10.2166/wpt.2013.037.

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