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Distribution of *Escherichia coli*, coliphages and enteric viruses in water, epilithic biofilms and sediments of an urban river in Germany

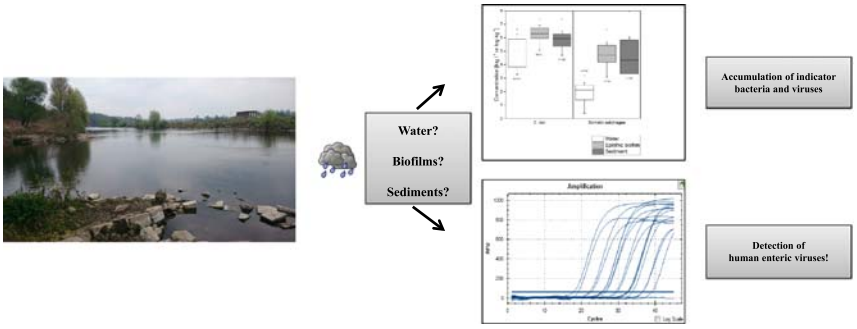
Martin Mackowiak [a,c,*], Mats Leifels [b,c,1], Ibrahim Ahmed Hamza [b,2], Lars Jurzik [b,c], Jost Wingender [a,c]

[a] University of Duisburg-Essen, Faculty of Chemistry, Biofilm Centre, Aquatic Microbiology, Essen, Germany
[b] Ruhr-University Bochum, Department of Hygiene, Social- and Environmental Medicine, Bochum, Germany
[c] Centre for Water and Environmental Research (ZWU), University of Duisburg-Essen, Essen, Germany

HIGHLIGHTS

- Enhanced rainfall promotes contamination of river water with fecal bacteria and viruses.
- *Escherichia coli*, coliphages and enteric viruses occurred in surface water biofilms and sediments.
- Adenoviruses were most prevalent in water, biofilms and sediments.
- River biofilms and sediments are reservoirs for microbial pathogens with a potential risk to public health.

GRAPHICAL ABSTRACT



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ABSTRACT

Fecal contamination of surface water is commonly evaluated by quantification of bacterial or viral indicators such as *Escherichia coli* and coliphages, or by direct testing for pathogens such as enteric viruses. Retention of fecally derived organisms in biofilms and sediments is less frequently considered. In this study, we assessed the distribution of *E. coli*, somatic coliphages, and enteric viruses including human adenovirus (HAdV), enterovirus (EV), norovirus genogroup GII (NoV GII) and group A rotavirus (RoV) in an urban river environment in Germany. 24 samples each of water, epilithic biofilms and sediments were examined. *E. coli* and somatic coliphages were prevalent not only in the flowing water, but also in epilithic biofilms and sediments, where they were accumulated compared to the overlying water. During enhanced rainfall, *E. coli* and coliphage concentrations increased by approximately 2.5 and 1 log unit, respectively, in the flowing water, whereas concentrations did not change significantly in epilithic biofilms and sediments. The occurrence of human enteric viruses detected by qPCR was higher in water than in biofilms and sediments. 87.5% of all water samples were positive for HAdV. Enteric viruses found less frequently were EV, RoV and NoV GII in 20.8%, 16.7% and 8.3% of the water samples, respectively. In epilithic biofilms and sediments, HAdV was found in 54.2% and 50.0% of the samples, respectively, and EV was found in 4.2% of both biofilm and sediment samples. RoV and NoV GII were not detected in any of the biofilms and sediments. Overall, the prevalence of enteric viruses was in the order of HAdV > EV > RoV ≥ NoV GII. In conclusion, epilithic biofilms and sediments can be reservoirs for fecal indicators and enteric viruses and thus should be taken into consideration when assessing microbial pollution of surface water environments.

* Corresponding author at: University of Duisburg-Essen, Faculty of Chemistry, Biofilm Centre, Aquatic Microbiology, Universitätsstraße 5, 45141 Essen, Germany.
E-mail address: martin.mackowiak@uni-due.de (M. Mackowiak).
[1] Present address: University of Alberta, School of Public Health, Edmonton, Canada.
[2] Present address: Environmental Virology Laboratory, Department of Water Pollution Research, National Research Centre, Cairo, Egypt.

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1. Introduction

The microbiological water quality of river water can be impacted by the input of fecally derived microorganisms form diverse sources including discharge of treated sewage from wastewater treatment plants, combined sewer overflows, surface runoff from urban and agricultural areas and wildlife (Rechenburg et al., 2006), but also from the mobilization of fecal organisms from the riverbed during sediment-disturbing events such as floods or heavy rainfall (Muirhead et al., 2004; Garcia-Aljaro et al., 2017). Contamination with fecal pathogens poses a risk to human health in surface waters used for recreation such as swimming, and results in the deterioration of microbiological quality of raw water abstracted for drinking production. One of the most important prerequisites in that respect is that the hygienic quality of the water is sufficient to prevent waterborne infections of humans. In a previous study, the microbiological water quality of the River Ruhr (Germany) flowing through an intensely industrialized region and impacted by discharge of treated sewage and combined sewer overflow events was monitored over an 18-month period (Strathmann et al., 2016). The study area included a stretch of this urban river including a barrier lake (Lake Baldeney) that was of particular interest for the implementation of bathing sites. A risk assessment showed that among enteric bacterial, viral and protozoan pathogens, the highest risk of acquiring gastrointestinal disease from bathing at selected sites of the river was presented by enteric viruses (noroviruses, rotaviruses) (Timm et al., 2016). The group of enteric viruses includes enteroviruses, hepatoviruses, adenoviruses, caliciviruses, astroviruses and reoviruses, mostly transmitted via the fecal-oral route. Besides viral gastroenteritis, some enteric viruses can cause hepatitis or illness of the central nervous system. Via raw sewage, viruses enter the wastewater treatment plant, where their concentration is reduced, but they are not completely eliminated (Jurzik et al., 2015), thus enteric viruses can be detected in surface waters with concentrations of up to 10^4 genome equivalents per liter (Hamza et al., 2009). The hygienic relevance results from the increased environmental stability compared to bacteria and the low infectious dose, but it is not known whether sediments or epilithic biofilms contribute to the risk of bathing in the River Ruhr. Biofilms are ubiquitous in aquatic environments, in technical and natural systems such as surface waters and they can act as reservoir for pathogenic microorganisms (Wingender, 2011). However, the interaction between viruses and biofilms has only been poorly investigated. Although human pathogenic viruses cannot multiply outside their host cells, they might accumulate in aquatic biofilms and sediments, where they could be protected against inactivation (Chung and Sobsey, 1993) and be released after a certain time into the water. The interaction between viruses and different types of aquatic biofilms was addressed in some studies; however, most of them focused on phages as surrogates for human enteric viruses (Helmi et al., 2008; Skraber et al., 2007; Storey and Ashbolt, 2001; Storey and Ashbolt, 2003). Only limited data are available showing that enterovirus, adenovirus and rotavirus species A seem to accumulate in river water sediments (Ali et al., 2004; Elmahdy et al., 2016; Garcia-Aljaro et al., 2017). The concentration of coliphages in surface waters does not necessarily correlate with that of human enteric viruses (Jiang and Chu, 2004; Jurzik et al., 2010) and their adsorption to sediments or biofilms depends on the type of virus (Botzenhart and Hock, 2002; LaBelle and Gerba, 1979). Thus, it is not clear whether phages could act as potential surrogates for hygienically relevant viruses in complex river environments.

Extending the monitoring study of Strathmann et al. (2016) that was based on the microbiological analysis of samples from the water column, here we focused on the distribution of bacteria and viruses both in bulk river water as well as in epilithic biofilms and sediments in the same area along the River Ruhr through a period of two months in the summer season. In this study, the occurrence of human enteric viruses, including adenoviruses, enteroviruses, noroviruses and rotaviruses, in water, sediment and epilithic biofilms in a river environment (River Ruhr, Germany) was investigated. All these viruses are mainly transmitted via the fecal-oral route (Gerba, 2009). Characteristics of the selected enteric viruses are shown in Table 1. In parallel, the occurrence of somatic coliphages, controversially discussed as potential surrogates for human enteric viruses, and of *E. coli* as a commonly used fecal indicator and in its function as a host for coliphages was assessed.

2. Material and methods

2.1. Viruses and bacteria

The somatic coliphage φX174 and *Escherichia coli* DSM 13127 were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Coliphages were propagated in *E. coli* DSM 13127 to prepare a stock suspension according to the standard DIN EN ISO 10705-2 (2000). As a human adenovirus (HAdV), an adenoviral vector based on human adenovirus type 5 was used and propagated to prepare stock suspensions of purified viruses as described before (Hamza et al., 2009). Murine norovirus was kindly provided by the Friedrich Loeffler Institute (Germany) and propagated in RAW 264.7 cells. After infection, cells were grown in RPMI 1640 medium supplemented with 2.5% fetal bovine serum at 37 °C under 5% CO_2 atmosphere for 5 days. Viruses were purified by centrifugation for 5 min at 300 ×*g* to remove cell debris and the supernatant was used as a stock suspension.

2.2. Sampling

In the period from July to September 2015, 24 samples each from water, epilithic biofilms and sediments were collected weekly over two months at three different sampling sites along the River Ruhr including a barrier lake (Lake Baldeney) in the City of Essen, a densely populated metropolitan area in North Rhine Westphalia, Germany (Fig. 1; SP1 ± 51°26′30.4″N 7°04′09.4″E, SP2 ± 51°24′22.0″N 7°01′11.2″E, SP3 ± 51°22′51.5″N 6°59′51.1″E). Sampling sites were chosen based on a previous study (Strathmann et al., 2016). The sampling points SP1 (Schwimmverein Steele) and SP3 (Löwental) were located upstream and downstream of Lake Baldeney, while SP2 (Seaside Beach) was located at Lake Baldeney. For each sampling site, 11 l of water, a sample of river sediment, and a sample of epilithic biofilm were collected. For sediments, a sample from the top layer was taken with a sterile metal spoon and transferred to a sterile conical tube. Macroscopically, sediments mainly consisted of sand and gravel, coarse fragments such as stones and wood bigger than approximately 1 cm in diameter were excluded from sampling. Epilithic biofilm was removed from stones using a sterile rubber spatula and transferred into sterile conical tubes at the sampling site. The collection of samples was approved by the German Federal Ministry of Education and Research (BMBF project number 02WRS1283A to J) and did not involve endangered or protected species. Water temperature was measured at the sampling site.

The samples were transported into the laboratory in <2 h under cooling and sample processing was conducted within the same day. Data for daily precipitation at the weather station in Essen-Bredeney were obtained online (Deutscher Wetterdienst, 2017). Additionally, average daily flow rate data from a site approximately 100 m upstream of SP3 were obtained online (Ruhrverband, 2016).

Table 1
Characteristics of the enteric viruses analyzed in this study (Gerba, 2009).

Viruses	Size	Genome	Clinical illness (examples)
Adenoviruses	70 nm	dsDNA	Gastroenteritis, respiratory diseases
Enteroviruses	27–32 nm	ssRNA	Encephalitis, meningitis, myocarditis
Rotaviruses	70 nm	dsRNA	Gastroenteritis
Noroviruses	26–35 nm	ssRNA	Gastroenteritis