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Quantitative microbial risk assessment of microbial source tracking markers in recreational water contaminated with fresh untreated and secondary treated sewage

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ABSTRACT

Microbial source tracking (MST) methods have provided the means to identify sewage contamination in recreational waters, but the risk associated with elevated levels of MST targets such as sewage-associated *Bacteroides* HF183 and other markers is uncertain. Quantitative microbial risk assessment (QMRA) modeling allows interpretation of MST data in the context of the risk of gastrointestinal (GI) illness caused by exposure to known reference pathogens. In this study, five sewage-associated, quantitative PCR (qPCR) MST markers [*Bacteroides* HF183 (HF183), *Methanobrevibacter smithii nifH* (*nifH*), human adenovirus (HAdV), human polyomavirus (HPyV) and pepper mild mottle virus (PMMoV)] were evaluated to determine at what concentration these nucleic acid markers reflected a significant health risk from exposure to fresh untreated or secondary treated sewage in beach water. The QMRA models were evaluated for a target probability of illness of 36 GI illnesses/1000 swimming events (i.e., risk benchmark 0.036) for the reference pathogens norovirus (NoV) and human adenovirus 40/41 (HAdV 40/41). Sewage markers at several dilutions exceeded the risk benchmark for reference pathogens NoV and HAdV 40/41. HF183 concentrations 3.22×10^3 (for both NoV and HAdV 40/41) gene copies (GC)/100 mL of water contaminated with fresh untreated sewage represented risk > 0.036. Similarly, HF183 concentrations 3.66×10^3 (for NoV and HAdV 40/41) GC/100 mL of water contaminated with secondary treated sewage represented risk > 0.036. HAdV concentration as low as 4.11×10^1 GC/100 mL of water represented risk > 0.036 when water was contaminated with secondary treated sewage. Results of this study provide a valuable context for water quality managers to evaluate human health risks associated with contamination from fresh sewage. The approach described here may also be useful in the future for evaluating health risks from contamination with aged or treated sewage or feces from other animal sources as more data are made available.

1. Introduction

Humans may be exposed to a variety of disease-causing microorganisms in recreational waters. A meta-analysis found that the risk of contracting diarrhea for non-swimmers was 35/1000, which rose to 59/1000 after swimming at beaches where the fecal indicator bacteria (FIB) *Enterococcus* spp. exceeded 35 CFU/100 mL of water (Arnold et al., 2016). Fecal pathogens can cause diarrhea, abdominal pain, cramping, nausea, and vomiting in healthy humans. Among sources of fecal contamination, human feces, including untreated or improperly treated sewage and septage, generally represents a greater risk to human health than contamination from animal feces, largely due to the presence and greater abundance of human-specific enteric viruses (Soller et al., 2010a). Furthermore, enteric viruses have low median infectious doses, resulting in the potential for substantial health risks in healthy populations (Haas et al., 1993). However, fecal contamination from some animals, such as cattle, may also pose a significant human health risk (Soller et al., 2010a).

Since sewage contamination poses greater risks to recreational water users than does animal fecal pollution, it is important to identify

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the sources of such contaminants in recreational water so that appropriate mitigation strategies can be implemented, and risks can be accurately assessed (Soller et al., 2010a). However, based on concentrations of FIB, it is not possible to attribute the sources to human and specific animal hosts because FIB are excreted in the feces of all warm-blooded animals. To overcome this limitation, researchers have developed a range of quantitative PCR (qPCR) assays to quantify host-associated molecular markers in environmental waters. These methods and others have been referred to as microbial source tracking (MST) tools.

Sewage-associated molecular markers in current use for MST are *Bacteroides* HF183 (Green et al., 2014), BacHum-UCD (Kildare et al., 2007), HumM2 (Shanks et al., 2009), *Methanobrevibacter smithii nifH* (Johnston et al., 2010; Ahmed et al., 2012), human adenovirus A-F (HAdV; designated as a marker in this study) (Rusiñol et al., 2014), human polyomaviruses (HPyV) (McQuaig et al., 2009; Ahmed et al., 2010) and pepper mild mottle viruses (PMMoV) (Rosario et al., 2009). While the presence and concentrations of these markers in a water body provide information regarding potential sources, and perhaps the magnitude of the issue (i.e., high or low levels of contamination), it is difficult to interpret qPCR-generated marker concentration data in terms of human health risks (Boehm et al., 2015; Wang et al., 2013).

Staley et al. (2012) linked the HF183 and HPyV markers to human health risk in recreational waters by determining the process limit of detection (PLOD) in dilutions of fresh untreated sewage and estimating the risk of gastroenteritis from exposure to NoV in the same diluted sewage. Both the HF183 and HPyV markers were detectable in water samples from several sites containing diluted sewage, and the probability of illness (P_{ill}) exceeded a 10/1000 benchmark, based on a quantitative microbial risk assessment (QMRA) analysis. HF183 was also detectable in water samples containing diluted sewage where human health risk was below the 10/1000 benchmark, suggesting a low concentration of this marker may not pose a meaningful risk to recreational water users. The HPyV marker, which is less concentrated in fresh untreated sewage than is HF183, was not detected in diluted water samples seeded with sewage compared to the HF183, leading the authors to conclude that the HPyV marker may not provide adequate public health protection when used as the sole indicator of sewage contamination (Staley et al., 2012). A similar QMRA approach was undertaken in a recent study for the PMMoV marker in Florida (Symonds et al., 2016). The risk benchmark was set to 0.036 (36 GI illnesses/1000 people). Based on the PMMoV limit of detection, it was found that at a dilution of 10^{-2}, the median GI illness risk was > 0.036.

A recent study also used a QMRA approach to simulate the risk of GI illness associated with swimming in waters containing different concentrations of HF183 and HumM2 markers (Boehm et al., 2015). The volume/volume ratio of untreated sewage to ambient water was determined by comparing marker concentrations in recreational water to untreated sewage across the USA. The results indicated that median concentrations of 4.20×10^3 and 2.80×10^3 GC of HF183 and HumM2 markers/100 mL, respectively, in recreational water will surpass a risk benchmark of 30 GI illnesses/1000 swimmers/swimming event. The study by Boehm et al. (2015) is the first to establish a risk-based approach for interpreting concentrations of HF183 and HumM2 markers in ambient waters. This is particularly important because the concentrations of sewage-associated markers can be translated into a health risk. However, such data (i.e., marker concentration threshold) are not available for other sewage-associated markers such as *nifH*, HAdV, HPyV, and PMMoV. Furthermore, these QMRA estimates were done in the USA, and limited information is available from other countries where these markers are frequently used to detect sewage contamination of waterways. In addition, these studies used fresh untreated sewage as analytical material, thus the GI risk for a scenario where the water is contaminated with secondary treated sewage is not known.

The main objective of this study was to undertake an exploratory QMRA analysis based on the process limit of quantification (PLOQ) of five sewage-associated MST markers targeting HF183, *nifH*, HAdV, HPyV and PMMoV in beach water samples seeded with fresh untreated and secondary treated sewage samples in Australia. Subsequently, concentrations of sewage markers that would pose a human health risk were estimated. The current study will enhance our understanding of the interpretation of sewage marker concentration data in the context of risk identification and mitigation.

2. Materials and methods

2.1. Determination of PLOQ for sewage-associated MST markers

A previous study determined the PLOQ values of six sewage-associated markers targeting *Escherichia coli* (EC) H8, *Bacteroides* HF183, *M. smithii nifH*, HAdV, HPyV and PMMoV (Hughes et al., 2017). For this study, the *Bacteroides* HF183, *M. smithii nifH*, HAdV, HPyV and PMMoV markers were chosen, while EC H8 was omitted. The latter, EC H8 marker, and identical sequences can be found in genera like *Yersinia* and *Klebsiella*, thereby compromising host-specificity (Ahmed et al., 2015). Briefly, to determine the PLOQ of the sewage-associated nucleic acid markers (HF183, *nifH*, HAdV, HPyV and PMMoV), 3 mL of untreated sewage was seeded into 297 mL of filter-sterilized beach water samples (at a salinity of 34‰), in triplicate (Hughes et al., 2017). Similarly, 150 mL of secondary treated sewage was seeded into 150 mL of the filtered beach water samples, in triplicate. Tenfold serial dilutions (10^{-1} to 10^{-6}) of all sewage-seeded samples were then made prior to analyses. The samples were filtered through negatively charged 47-mm, 0.45-μm-pore-size HA membranes (Merck Millipore, Tokyo, Japan). Nucleic acid samples were extracted directly from the membranes using the Mo Bio PowerWater DNA and RNA isolation kits (Mo Bio Laboratories, Carlsbad, CA, USA). The qPCR analyses including primers and probes, chemistry and cycling parameters have been described elsewhere in detail (Hughes et al., 2017). During qPCR analysis, all DNA and RNA samples were run in triplicate with three negative controls (sterile water) on 96-well plates using the CFX 96 thermocycler (Bio-Rad Laboratories, CA, USA) (Hughes et al., 2017).

2.2. Process limit of quantification (PLOQ)

Defined criteria were established to determine the PLOQ. The PLOQ was defined as the smallest volume of sewage that could be subjected to the complete sample preparation process, including dilution, filtration, and nucleic acid extraction and still be reliably quantified in 2/3 qPCR reactions (Staley et al., 2012; Symonds et al., 2016).

2.3. Quantitative microbial risk assessment

NoV and HAdV 40/41 were selected as reference pathogens as these viruses are known to cause swimming-associated illnesses in recreational waters (Zlot et al., 2015; Kauppinen et al., 2017). Use of reference pathogens is an accepted practice in the field of QMRA (Soller et al., 2006; Soller et al., 2010a; Schoen et al., 2017). Furthermore, information such as concentrations of NoV and HAdV in sewage (Pina et al., 1998; Eftim et al., 2017; Hughes et al., 2017), and dose-response models exist in the literature (Crabtree et al., 1997; Teunis et al., 2010; Teunis et al., 2016) making it possible to assess human health risks.

The concentrations of NoV and HAdV 40/41 in untreated sewage and secondary treated sewage were obtained from our previous study (Hughes et al., 2017). NoV concentrations data in untreated sewage produced a mean and standard deviation of $9.66 \times 10^5 \pm 2.85 \times 10^5$ gene copies (GC)/L. A lognormal distribution with parameters ($\mu = 13.7$, $\sigma = 0.28$) was used calculate the dose (Table 1). The HAdV 40/41 concentration data in untreated sewage produced a mean and standard deviation of $9.66 \times 10^5 \pm 2.85 \times 10^5$ gene copies (GC)/L.

A lognormal distribution with parameters ($\mu = 14.7$, $\sigma = 0.67$) was used to calculate the dose.