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Comparison of molecular markers to detect fresh sewage in environmental waters

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ABSTRACT

Human-specific *Bacteroides* HF183 (HS-HF183), human-specific *Enterococci faecium esp* (HS-*esp*), human-specific adenoviruses (HS-AVs) and human-specific polyomaviruses (HS-PVs) assays were evaluated in freshwater, seawater and distilled water to detect fresh sewage. The sewage spiked water samples were also tested for the concentrations of traditional fecal indicators (i.e., *Escherichia coli*, enterococci and *Clostridium perfringens*) and enteric viruses such as enteroviruses (EVs), sapoviruses (SVs), and torquetenoviruses (TVs). The overall host-specificity of the HS-HF183 marker to differentiate between humans and other animals was 98%. However, the HS-*esp*, HS-AVs and HS-PVs showed 100% host-specificity. All the human-specific markers showed >97% sensitivity to detect human fecal pollution. *E. coli*, enterococci and, *C. perfringens* were detected up to dilutions of sewage 10^{-5}, 10^{-4} and 10^{-3} respectively. HS-*esp*, HS-AVs, HS-PVs, SVs and TVs were detected up to dilution of sewage 10^{-4} whilst EVs were detected up to dilution 10^{-5}. The ability of the HS-HF183 marker to detect fresh sewage was 3–4 orders of magnitude higher than that of the HS-*esp* and viral markers. The ability to detect fresh sewage in freshwater, seawater and distilled water matrices was similar for human-specific bacterial and viral marker. Based on our data, it appears that human-specific molecular markers are sensitive measures of fresh sewage pollution, and the HS-HF183 marker appears to be the most sensitive among these markers in terms of detecting fresh sewage. However, the presence of the HS-HF183 marker in environmental waters may not necessarily indicate the presence of enteric viruses due to their high abundance in sewage compared to enteric viruses. More research is required on the persistency of these markers in environmental water samples in relation to traditional fecal indicators and enteric pathogens.

1. Introduction

Fecal pollution is one of the major concerns in relation to water bodies used for drinking water supply, recreational activities and harvesting seafood due to likely exposure to a wide array of pathogenic bacteria, protozoa and viruses (Hörman et al., 2004; Fong and Lipp, 2005). Various sources such as agricultural run-off, wild animals, combined sewer overflows (CSOs), sewage treatment plants (STPs), defective on-site wastewater treatment systems and industrial wastewater outlets are known to be potential sources of such pollution. The microbiological quality of water is generally assessed by enumerating fecal indicator bacteria such as *Escherichia coli* and enterococci which are commonly found in

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the feces of warm-blooded animals including humans (USEPA, 2000). The presence of these indicators in water bodies generally points to fecal pollution and potential public health risks. The identification of indicator bacteria from major polluting source(s) is vitally important in order to implement appropriate mitigation strategies to minimise fecal pollution and associated public health risks (Scott et al., 2002). However, the assignment of indicator bacteria to human and animal sources in environmental waters is difficult due to their cosmopolitan nature (Field and Samadpour, 2007). In addition, environmental waters can be impacted by multiple sources of fecal pollution making it extremely difficult to implement a robust management plan without understanding the potential sources of pollution.

Over the last decade, microbial source tracking (MST) techniques have been developed to distinguish human from animal fecal pollution. The underlying assumption of MST is that the host-specificity of microorganisms is influenced by selective pressure in the host animal gut (Wiggins, 1996). The majority of the early MST methods are library-dependent which require the development of a collection of *E. coli* or enterococci isolates from suspected sources using various phenotypic and genotypic methods. Phenotypic or genotypic patterns of target strains are then compared to the library to identify their likely sources (Scott et al., 2002). There are several significant limitations in library-dependent methods which have been widely reported in the research literature such as: (1) a large representative library is required for successful field application. The development of such a library is laborious, and usually costly when using phenotypic and genotypic methods (i.e., PFGE and carbon source utilization) (Field and Samadpour, 2007); (2) commonly used fecal indicator bacteria (*E. coli* and/or enterococci) lack host-specificity (Gordon et al., 2002); (3) a library consisting of a small number of isolates cannot be readily used in multiple catchments, and therefore development of a separate library may be required for each catchment of interest (Ahmed et al., 2006; Hartel et al., 2002; Scott et al., 2003), and (4) library-dependent methods may yield both high false positive and negative results (Harwood et al., 2003; Moore et al., 2005).

Certain limitations of library-dependent methods could be overcome by using library-independent methods. These methods rely on detecting host-specific molecular markers in a given environmental sample using PCR assays. These methods are rapid and have shown to have higher specificities in a method comparison study (Griffith et al., 2003). The most commonly used markers for MST can be categorised into three groups: (1) anaerobic bacterial markers (i.e., host-specific *Bacteroides* PCR) (Bernhard and Field, 2000), (2) bacterial toxin markers (i.e., *E. faecium esp* and *E. coli* toxin gene markers) (Scott et al., 2005; Khatib et al., 2002), and (3) viral markers (i.e., host-specific adenoviruses and polyomaviruses) (Fong and Lipp, 2005; McQuaig et al., 2006). Several studies have reported high host specificities of these markers which makes them suitable to distinguish between sources of fecal pollution (Ahmed et al., 2008a; Seurinck et al., 2005; Bernhard and Field, 2000; Reischer et al., 2006; Scott et al., 2005; Khatib et al., 2002).

Library-independent methods also have potential limitations, including the detection of certain markers in a small number of non-target samples (Gourmelon et al., 2007; Carson et al., 2005; Gawler et al., 2007; Whitman et al., 2007). Another limitation of these markers is that they are not present in the feces of all individuals, and the concentrations may vary from one DNA target to another (Field and Samadpour, 2007). For example, the concentration of human-specific *Bacteroides* markers in sewage samples could be 4–5 orders of magnitude higher than human-specific viral or toxin gene markers. Moreover, little is known regarding the persistency of these markers in environmental waters. In addition, the correlation between some of these markers with traditional fecal indicators and pathogens is not well documented. The absence of a particular marker in environmental waters does not completely rule out the presence of fecal pollution from that particular source. A general consensus is that multiple markers should be used (where possible) to obtain accurate and confirmatory results. To-date, only a few studies have used multiple host-specific markers to identify the sources of fecal pollution in environmental waters (Ahmed et al., 2007; Gourmelon et al., 2007; McQuaig et al., 2006). These markers appear to be promising in identifying the sources of fecal pollution. However, more research is required prior to their application for routine monitoring of water quality. A recent review paper highlighted the various research gaps that need to be addressed for library-independent methods (Santo Domingo et al., 2007).

The aim of this study was to evaluate the human-specific *Bacteroides* HF183 (HS-HF183), human-specific *E. faecium esp* (HS-*esp*), human-specific adenoviruses (HS-AVs), and human-specific polyomaviruses (HS-PVs) markers to detect the smallest amount of fresh sewage pollution in sewage spiked freshwater, seawater and distilled water samples using real-time PCR assays. Furthermore, the sewage spiked water samples were also tested for the concentrations of fecal indicators such as *E. coli*, enterococci and *Clostridium perfringens*. In addition, real-time PCR assays were also used to detect enteric viruses such as emerging enteroviruses (EVs), sapoviruses (SVs), and torquetenoviruses (TVs) for sewage spiked water samples. These enteric viruses are excreted in extremely high numbers in the feces of infected individuals and can cause mild to severe gastroenteritis in humans. Humans could be exposed to enteric viruses by using contaminated waters for shellfish harvesting or recreation, or as a source of drinking waters. Fecal indicators' concentrations and the ability of each human-specific marker to detect fresh sewage were used to obtain a better understanding of which fecal indicators and human-specific marker(s) could potentially indicate the presence of enteric viruses in environmental waters polluted with fresh sewage.

2. Materials and methods

2.1. Host-specificity and sensitivity of human-specific markers

Host-specificity and sensitivity are commonly used parameters for human-specific markers. The specificity of a marker is the proportion of negative-control samples in which the marker is detected and the sensitivity of a marker is the