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Relationship of human-associated microbial source tracking markers with Enterococci in Gulf of Mexico waters

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ABSTRACT

Human and ecosystem health can be damaged by fecal contamination of recreational waters. Microbial source tracking (MST) can be used to specifically detect domestic sewage containing human waste, thereby informing both risk assessment and remediation strategies. Previously, an inter-laboratory collaboration developed standardized PCR methods for a bacterial, an archaeal, and a viral indicator of human sewage. Here we present results for two subsequent years of field testing in fresh and salt water by five laboratories across the U.S. Gulf Coast (two in Florida and one each in Mississippi, Louisiana and Texas) using common standard operating procedures (SOPs) developed previously. Culturable enterococci were enumerated by membrane filtration, and PCR was used to detect three MST markers targeting domestic sewage: human-associated *Bacteroides* (HF183), *Methanobrevibacter smithii* and human polyomaviruses BK and JC (HPyVs). Detection of sewage markers in surface waters was significantly associated with higher enterococci levels and with exceedance of the recreational water quality standard in four or three regions, respectively. Sewage markers were frequently co-detected in single samples, e.g., *M. smithii* and HF183 were co-detected in 81% of Louisiana samples, and HPyVs and *M. smithii* were co-detected in over 40% of southwest Florida and Mississippi samples. This study demonstrates the robustness and inter-laboratory transferability of these three markers for the detection of pollution from domestic sewage in the waters impacting the Gulf of Mexico over a coastal range of over 1000 miles.

1. Introduction

The waters of the Gulf of Mexico have an appreciable impact on the economies of bordering states. For example, saltwater fishing alone has an estimated economic impact of $8 billion annually (Theodore Roosevelt Conservation Partnership, 2011). However fecal contamination by point and non-point sources can negatively impact water quality, tourism, fishing and shellfishing industries, causing economic losses and environmental damage. Rapid identification and remediation

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of the source of contamination can minimize the impacts of such pollution on the economy, public health, and ecosystem health. However, the fecal indicator bacteria (FIB) such as *Escherichia coli* and enterococci used as surrogates for pathogens in water quality monitoring (U.S. Environmental Protection Agency, 1978; U.S. Environmental Protection Agency, 2004) are present in the feces of warm-blooded animals including humans and some cold-blooded animals (Harwood et al., 1999; Habersack et al., 2011; Souza et al., 1999), providing no information about pollution sources.

Human fecal material poses a known human health risk if introduced, untreated, into the environment as it contains a variety of pathogens including bacteria, protozoa and viruses, some of which are human-specific (Girones et al., 2010; Harwood et al., 2005; Lee et al., 2008). This disparate assortment of pathogens, represented by microbes of widely varying morphology, physiology, and ecology, is expected to show varying responses to environmental stresses and have differing transport characteristics and effects of competition and predation. Thus, the indicator paradigm of one FIB to indicate all types and sources of fecal pollution may not be the optimal approach to water quality regulation (Harwood et al., 2005).

The current EPA guidelines for recreational water quality are based on epidemiology studies in which gastrointestinal illness in swimmers at beaches impacted with point source discharges of municipal sewage were correlated with enterococci levels (Cabelli et al., 1982; Dufour, 1984). However, several subsequent studies have shown no correlation between FIB levels and gastroenteritis in waters contaminated by nonpoint sources, such as stormwater runoff (Colford et al., 2007; Calderon et al., 1991; Sinigalliano et al., 2010). In light of recent developments in rapid detection methods, the EPA has proposed new optional recreational water quality monitoring methods which use rapid quantitative PCR in 2012 (U.S. Environmental Protection Agency, 2012) to better protect human health by providing water quality information in a more timely manner to the public.

The field of microbial source tracking (MST) has emerged in an effort to identify the dominant source(s) of fecal pollution in environmental waters (Field and Samadpour, 2007; Hagedorn et al., 2011; Stoeckel and Harwood, 2007). MST methods have tended to focus on relatively rapid and highly specific library-independent methods such as PCR for source-specific gene targets. Such approaches potentially allow the public and beach managers to be more quickly informed of a possible health risk due to elevated FIB levels in recreational waters, as well as aiding local and state agencies responsible for remediation efforts by providing contamination source information (Propst et al., 2011). Investigators have identified source-specific-bacterial groups including the order Bacteroidales (Bernhard and Field, 2000), Archaea such as *Methanobrevibacter smithii* (Harwood et al., 2009; Ufnar et al., 2006) and viruses such as bacteriophages, adenoviruses and human polyomaviruses (HPyVs) (Ahmed et al., 2009; Gourmelon et al., 2007; Harwood et al., 2009; Kirs and Smith, 2007; McQuaig et al., 2009; Stewart-Pullaro et al., 2006; Wolf et al., 2010) as targets for PCR-based methods to detect domestic sewage contamination.

In an effort to include a phylogenetically, physiologically, and morphologically-diverse microbial group, a toolbox of human-associated MST targets representing *Bacteria*, *Archaea* and viruses (human-associated *Bacteroides*, *M. smithii* and human polyomaviruses BK and JC, respectively) were tested in this study. The human-associated *Bacteroides*, HF183, have been extensively studied by PCR and quantitative PCR (QPCR) (Bernhard and Field, 2000; Kildare et al., 2007; Layton et al., 2006; Reischer et al., 2007; Seurinck et al., 2005; Shanks et al., 2009). The *Bacteroidales* are dominant members of intestinal microflora in many animals, including humans and were correlated with the presence of pathogenic enteric bacteria in environmental waters (Walters et al., 2007). *M. smithii* is reportedly specific to the human gastrointestinal tract and, as a member of the *Archaea*, is phylogenetically distant from the bacterial and viral targets (Ufnar et al., 2006). Positive associations have been observed between FIB concentration or human pathogen presence and HPyVs in the environment (Korajkic et al., 2011; McQuaig et al., 2006, 2009). Furthermore, HPyVs may better mimic the transport and survival characteristics of human viral pathogens than bacteria.

The data presented here were collected as a continuation (years two and three) of a multi-laboratory study to standardize, validate and field test culture-independent and library-independent MST methods in order to determine whether these methods may be useful to regulators and managers of recreational water quality across the U.S. (Harwood et al., 2009). Five laboratories sampled Gulf of Mexico estuarine and coastal waters and tributary fresh wasters 100 miles or more in distance from one another in Florida (central peninsula and western panhandle), Mississippi, Louisiana and Texas. Following dissemination of standard operating procedures (SOPs), and demonstration of analytical capability by each laboratory, field testing was initiated for sewage markers (HF183, HPyVs and *M. smithii*) and enterococci. Enterococci concentrations were compared with detection of each of the three MST markers in ambient water samples to identify relationships between enterococci concentrations and MST marker presence. In addition, surface waters impacted by recent sewage contamination were sampled when local agencies notified laboratories of sewage spills that impacted a water body.

2. Materials and methods

Five university laboratories, each representing a different region, participated in this study, University of South Florida (USF) in Tampa, FL; University of West Florida (UWF) in Pensacola, FL; University of Southern Mississippi (USM) in Hattiesburg, MS; Nicholls State University (NSU) in Thibodaux, LA and Texas A&M University-Corpus Christi (TAMUCC) in Corpus Christi, TX (Mott). Each laboratory sampled coastal and estuarine waters in its immediate geographic vicinity, except that USM sampling was focused around Gulfport, MS (Table S1, Supplementary Materials). The SOPs developed previously (detailed below) were used to test the sensitivity and specificity of the markers in a geographically diverse set of sewage and fecal samples during the first year of this study at three of the laboratories, USF, USM and UWF (Harwood et al., 2009). Prior to beginning field sampling in the second year of the study, SOPs were distributed to all five laboratories