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Current and future trends in fecal source tracking and deployment in the Lake Taihu Region of China

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ABSTRACT

The emerging discipline of microbial and/or chemical source tracking (collectively termed fecal source tracking (FST)) is being used to identify origins of fecal contamination in polluted waters in many countries around the world. FST has developed rapidly because standard methods of measuring contamination in water by enumerating fecal indicator bacteria (FIB) such as fecal coliforms and enterococci do not identify the sources of the contamination. FST is an active area of research and development in both the academic and private sectors and includes:

- Developing and testing new microbial and chemical FST methods.
- Determining the geographic application and animal host ranges of existing and emerging FST techniques.
- Conducting experimental comparisons of FST techniques.
- Combining direct monitoring of human pathogens associated with waterborne outbreaks and zoonotic pathogens responsible for infections among people, wildlife, or domesticated animals with the use of FST techniques.
- Applying FST to watershed analysis and coastal environments.
- Designing appropriate statistical and probability analysis of FST data and
- developing models for mass loadings of host-specific fecal contamination.

This paper includes a critical review of FST with emphasis on the extent to which methods have been tested (especially in comparison with other methods and/or with blind samples), which methods are applicable to different situations, their shortcomings, and their usefulness in predicting public health risk or pathogen occurrence. In addition, the paper addresses the broader question of whether FST and fecal indicator monitoring is the best approach to regulate water quality and protect human health. Many FST methods have only been tested against sewage or fecal samples or isolates in laboratory studies (proof of concept testing) and/or applied in field studies where the "real" answer is not known, so their comparative performance and accuracy cannot be assessed. For FST to be quantitative, stability of ratios between host-specific markers in the environment must be established. In addition, research is needed on the correlation between host-specific markers and pathogens, and survival of markers after waste treatments. As a result of the exclusive emphasis on FIB by regulatory agencies, monitoring and FST development has concentrated on FIB rather than the actual pathogens. A more rational approach to regulating water quality might be to use available epidemiological data to identify pathogens of concern in a particular water body, and then use targeted pathogen monitoring coupled with very specific FST approaches to control the pathogens. Baseline monitoring of FIB would be just one tool among many in this example.

1. Introduction

Advances in FST have largely been driven by the need to comply with water quality standards based on the traditional FIB, as mandated by regulatory agencies (USEPA, 2007). Recently, a number of culture-independent, and library-independent methods based on

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polymerase chain reaction (PCR) have been gaining popularity among FST researchers. However, only a limited number of these methods have been successfully used in field applications, primarily because most are still under development. Different viewpoints associated with the practical use of FST have been published that identify critical research gaps, propose priority-based timelines to address them, and outline emerging technologies that will likely impact the future of FST (Tourlousse et al., 2008). It will be necessary to consider each of these aspects in order to advance towards a unifying framework in source identification, so that fecal pollution monitoring can be reliably used for comprehensive environmental microbial monitoring, to develop risk assessment models, and to implement and validate adequate management practices to reduce pollutant loading in receiving waters.

Two major classes of FST methods have been developed and widely used in water quality projects around the world (Blanch et al., 2006). Known as library-dependent and library-independent approaches, each currently possesses both strengths and weaknesses for source identifications. Library-dependent methods rely on the construction of a host-origin database (library) of isolates from known fecal sources. Bacterial isolates collected from these known fecal sources are assayed to provide a collection of 'fingerprint' patterns that allow for a direct comparison with the fingerprints of isolates of unknown origin (water isolates) using statistical classification algorithms. Most library methods are culture-based, and require growing environmental isolates from water samples (Atherholt, 2005). Library-dependent methods include both phenotypic and genotypic tests. Library-independent methods may be either culture-dependent or culture-independent. Culture-dependent, library-independent methods are based on growing source-specific viruses or bacteria. Library-independent, culture-independent methods include a variety of chemical and molecular tests where either DNA or a chemical is detected directly from water samples (Devereux et al., 2006).

FST is an evolving field that has undergone rapid diversification since the first approaches were described only a decade ago (Simmons et al., 1995). Since then, there have been three large multi-laboratory method comparison studies, (Blanch et al., 2006; Griffith et al., 2003; Stoeckel et al., 2004), research projects that included method comparisons (Noble et al., 2003; Vogel et al., 2007), numerous workshops dedicated to FST (Atherholt, 2005; Devereux et al., 2006; Pond et al., 2004), numerous review articles (Field and Brodeur, 2003; Field and Samadpour, 2007; Meays et al., 2004; Scott et al., 2002; Seurinck et al., 2005) and a federal guidance document (USEPA, 2005). More recently, Stoeckel and Harwood (2007) proposed a standardized approach that included performance criteria plus recommendations for field study design. These various documents combined provide a large body of guidance on FST, and the following sections include a summary of these sources that can serve as a model for researchers desiring to participate in the rapidly-developing field of FST.

2. FST microbes and methods

The initial step is performing FST is to choose a microorganism to work with. Even if the choice is to use the culture-independent and library-independent approach, it will still be necessary to become familiar with the growth and characteristics of the microbes that will be the source of the DNA. The majority of studies to date have used *E. coli* and *Enterococcus*, but many additional microbes are now being tested and evaluated for their suitability in FST (Tables 1 and 2).

FST involves selecting methods as well as microbes, and there is much variety in the choice of methods. Table 3 summarizes those methods most frequently reported in the literature, but is not a comprehensive list. Even with all of the publications on FST, the selection of microbes and methods for the researcher just beginning to work in FST can be difficult and confusing (Devereux et al., 2006).

As well as methods that use microorganisms, there are also chemicals that are specific to human wastewater and appear to offer several potential advantages over biologically based methods (Hagedorn and Weisberg, 2009). These chemical-based methods

Table 1
Bacteria commonly used in FST research.

Bacteria	Advantages	Disadvantages
Total/fecal coliforms	Used extensively as fecal indicators	Ecology, prevalence, resistance to stress differ from pathogens
E. coli	Not usually pathogenic to humans Present at concentrations higher than pathogens	May not be a good indicator in tropical/subtropical environments
Enterococcus	Especially useful in marine environments and recreational waters	Found in environmental reservoirs Regrowth possible
Bacteroides/Bifido	Less common in animals Human isolates ferment sorbitol Evidence of recent contamination	Survivability in environment is variable Culture methods not well defined
Clostridium perfrigens	Good for prediction of viruses or remote fecal pollution	Persistent in the environment

Table 2
Phages and viruses commonly used in FST research.

Virus	Advantages	Disadvantages
Bacteroides fragillis bacteriophage	Abundant in human feces Phages do not replicate in environment Presence correlates with presence of human enteric viruses	Phage found to be absent in some highly polluted environments
F-specific RNA coliphage	Groups I and II associated with human feces, group IV associated with animal feces	Sensitive detection methods required
	Easy to perform Rapid detection	Only small percentage of human feces contain phages Unreliable in marine waters
Human enteric viruses	Human specific No need to detect indicators	Low numbers in environment Over 120 enteric viruses