

Microbial Source Tracking to Identify Fecal Pollution Sources in Water

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Fecal pollution in aquatic systems is a significant problem in many areas of the world and there remains a need for techniques that can help to differentiate contaminant sources. Identifying sources of fecal pollution in water is an important step towards understanding the linkages between land-use and water quality, as well as providing evidence to inform decision making regarding effective mitigation strategies in a watershed.

Microbial source tracking (MST) is one technique that can aid in fecal source tracking. This technique uses microorganisms to determine the source of fecal pollution in the environment and has been used to understand contamination in drinking water sources and recreation areas, and for supporting watershed investigations [1, 2; 3, 4]. Many methods and techniques exist for MST and currently no standard tool exists. Choosing a MST method should be based on the expectations and needs of a specific study. The limitations of these techniques should also be carefully considered upon study development. Similar to other track-down methods, MST techniques should be used in combination with other investigative process, including local land-use information, to more accurately identify sources of fecal contamination in the environment [1, 5].

Quantifying the presence of fecal indicator bacteria, commonly *Escherichia coli* (*E. coli*) and *Enterococcus*, is the standard by which the extent of fecal pollution and the potential human health risk is currently assessed in environmental waters. Through routine detection, the occurrence of these traditionally used fecal indicator bacteria cannot be used to discriminate between sources due to their widespread distribution in the feces of humans and other animals, including wild and domestic animals. Increased evidence of environmental reservoirs further limits their connection to fecal sources [2, 6].



Figure 1. Schematic of potential microbial sources to watersheds

Many tools and techniques have been developed for MST over the years. With increased access to molecular detection equipment in many laboratories and a greater understanding of host-microbe ecology, the development of MST methods has accelerated in the past few decades.

In the 1970's, methods for source tracking included comparing ratios of different types of fecal bacteria in water, as different ratios exist in the feces of human and animal hosts [7]. However, as a result of differing environmental survival times of these bacteria, the ratios observed in water do not always reflect those found in an animal host. As a result this technique is no longer considered a valid tool for MST [8].

In the 1990's MST began to evolve rapidly and various methods were introduced into the literature. Some MST tools developed to include phenotypic comparisons, including physical or biochemical-based methods. These techniques included methods, such as antibiotic resistance profiling and carbon utilization, that compare phenotypic patterns in microbes from known host-sources and those found in the environment [3]. These techniques tend to be used with less frequency as most laboratories have moved towards molecular-based analysis.

Currently, most MST methods include molecular analysis. Molecular-based MST methods focus on detecting variations in the genetic material of a microbe or microbial population to determine the origin of fecal contamination in water. Other methods use molecular techniques to identify species or strains of microbes or viruses that are unique to a specific host. Some techniques are best suited to distinguish human versus non-human

contamination, whereas others can distinguish between multiple impacting fecal sources.

Molecular MST techniques can be differentiated into library and non-library (or library-independent) methods. Library-based molecular methods involve developing a database of genetic markers from microbes from known host sources, known as the library, and comparing these genetic patterns (or fingerprints) to those from microbes associated with contaminated water. Library-independent methods involve the detection of specific genetic markers in microbes with known host specificity or identification of species/strains of microbes that are common to a specific fecal source.

Library-based methods were initially developed for epidemiology studies to track down sources of disease causing microbes. After years of use, these techniques were adapted for environmental studies, and have been successfully used to understand fecal contamination in a variety of water sources around the world (see [5]).

Various techniques exist for library-dependant MST, including repetitive elements PCR (REP-PCR), pulsed field gel electrophoresis (PFGE), and ribotyping [1]. In general, these methods generate genetic patterns or fingerprints that are unique to bacteria isolated (e.g., *E. coli*) from various fecal sources in a watershed. The distinctive patterns generated in this library can then be compared to the isolates associated with fecal pollution in a water source. One advantage of library-dependant MST study is that multiple contaminant sources (e.g. humans, horses, dogs, pigs) can be identified within a watershed. Creation of a large library from various sources is important to the success of library-dependant MST studies; however,

these can be time intensive and costly to develop. A noteworthy disadvantage of these techniques is that the developed library is generally geographically limited, and cannot be used for source tracking in other areas, or even in the same watershed after a long period of time.

Recent studies in MST have focused on library-independent methods as these techniques tend to be less labor intensive, less expensive, and can be used across geographically diverse areas. Library-independent methods can involve the detection of host-specific genetic markers without the development of a library, such as Bacteroidales markers [4, 9, 10]. Library-independent MST techniques can also include identifying species or strains of microbes that are unique to a specific host animal, including human enteric viruses and *Cryptosporidium* [4, 11, 12]. Many of these MST techniques tend to be most useful to confirm the presence or absence of a particular source or understand the relative abundance of contamination from different sources.

Detection of host-specific markers in Bacteroidales has shown considerable promise among library-independent MST methods and increased use of this technique can be observed in the literature (e.g., [3, 4, 10]) Bacteroidales are a high density anaerobic gut bacteria that is highly adapted to living in specific host animals, which has resulted in genetic variation in these bacteria. Detecting these genetic variation in Bacteroidales found in contaminated water can help to identify the origin of these microbes. Unlike most library-dependant methods, Bacteroidales MST methods are culture-independent and markers can be detected through direct molecular analysis of water samples. This can be an advantage if rapid

results are needed, but can be a disadvantage if further characterization is desired. Bacteroidales MST can be used to identify human and non-human sources, although studies have begun to include a variety of other animal sources, including bovine, pig, elk and dog (see [4, 5]), to further define sources of fecal contamination in water.

Advancements in MST methods continue to develop. Techniques using advanced molecular analysis, including next-generation and high-throughput sequencing technologies are being applied to understand fecal sources and microbial diversity in streams. These techniques can rapid identify multiple markers of interest and allow for whole genome sequencing. With lower analysis cost in recent years, these powerful tools are becoming more commonly reported in MST literature (e.g., [13]).

Identifying sources of fecal contamination in aquatic systems can be challenging. MST can be a valuable tool to help understand and identify sources. While a standard MST method does not currently exist, various techniques have been developed and continue to be developed. Choosing a technique for a particular study should be based on the objectives of a study and limitations of these methods should be considered. Recognizing the sources of fecal pollution in water is an important step towards understanding how and why water quality impairments occur in a specific water course. This knowledge can aid in directing appropriate resources and providing thoughtful decision-making regarding targeted mitigation strategies to reduce future contamination.

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