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# Microbial Source Tracking and the TMDL (Total Maximum Daily Loads) Process

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## What Is Microbial Source Tracking?

Microbial Source Tracking (MST), also commonly referred to as bacterial source tracking (BST), is a method used to determine the sources of *fecal bacteria* and establish whether fecal bacteria are being introduced into water bodies through human, wildlife, agricultural, or pet wastes. MST is considered to be a novel technology still in developmental stages. However, the use of MST is rapidly becoming widespread as more researchers and states are recognizing its potential.

## MST Methods

There are four categories of methods currently being developed and used in MST: biochemical (phenotype), molecular (genotype), chemical, and immunological (the last two are not based on microbes, but are grouped with the microbe-based categories simply for convenience). Though all four categories have the same goal of identifying sources of fecal pollution, they each accomplish this goal with various degrees of labor and expense. Generally, one method will be more suitable for a specific task than another. In some cases, two methods may be used simultaneously to verify results. This practice is becoming more widely used, especially with combinations of methods from the different categories.

All methods in the first two categories (biochemical and molecular) first require the creation of a source library or database of host-origin or known sources such as human, cow, goose, etc. Source libraries are produced by collecting fecal samples and isolating fecal bacteria from them. When a fecal sample is collected, it is diluted, filtered onto a growth medium selective for the target fecal bacterium, and then incubated for 24 hours. At this time several colonies of bacteria will have grown, allowing lab personnel to separate or isolate the distinctive fecal bacteria from any other types of organisms. These separated colonies called isolates are then identified individually. After a source library is built, it can then be used to identify unknown-source fecal bacterial isolates gathered from an impaired water body by comparing the unknown isolates to those in the library.

### Biochemical

The biochemical method of MST is based on bacteria characteristics such as antibiotic resistance analysis (ARA). There are several other biochemical methods, but ARA has been the most widely used. Since humans, domestic animals, and wildlife are exposed to different antibiotics, fecal bacteria develop and express resistance to those different antibiotics. These distinctive resistances present in each contamination source make the fecal bacteria found in a contaminated water body reasonably unique to their source.

This method includes isolating fecal bacteria from water samples and then testing the bacteria on a range of different antibiotics. Bacteria from different sources will grow (or not grow) in a specific way for each

**fecal bacteria** - bacteria from the fecal coliform bacteria group originating in the intestinal tract of warm-blooded animals that passes into the environment in feces. Fecal coliform bacteria are often used as an indicator of pathogens in water.

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source, demonstrating a source-specific resistance pattern. This pattern is compared to a "source library" of patterns from bacteria that were isolated from specific hosts (e.g. humans, birds, cattle, deer, etc.) to determine which sources are causing water contamination. ARA is a fairly quick and inexpensive method but requires trained lab personnel to determine results.

### Molecular

The molecular method of MST is based on DNA patterns that are unique to each source due to variables such as the food consumption and health of the individual. As with the biochemical methods, fecal bacteria are isolated from water samples and a DNA pattern or "fingerprint" is obtained. The pattern is then compared to the library of patterns from specific sources to identify the source(s) of isolates in a sample.

There are over a dozen molecular methods currently under development; most require known-source libraries, but a few do not. Molecular methods are comparable to biochemical methods regarding identification of broad categories such as human vs. nonhuman, but are believed to be more accurate for individual sources (e.g. deer, muskrat, chickens, horses, etc.). However, the molecular methods are also considerably more expensive and slower to perform in the laboratory.

### Chemical

Chemical source-tracking methods are used to test water for various chemicals that are present as a result of human wastes. These chemicals can include laundry detergent, caffeine, and other source-specific fecal materials (e.g. sterols and stanols). These methods have been used mostly to determine if fecal bacteria from polluted waters were from human sources or not. Detection of optical brighteners from detergents has been the most widely used chemical method to date, and has been adopted by the Virginia Department of Health, Division of Shellfish Sanitation, to assess shellfish-bearing waters for human-derived sources of pollution. The fecal sterol method, while new and under development, does have the possibility of identifying sources from humans plus several other animals such as dogs, cattle, and birds.

### Immunological

Immunological source tracking methods are based on unique proteins called antigenic determinants that are shed in the fecal matter of humans and animals. Detection of these antigenic determinants by immunological procedures should allow reliable detection and the identification of specific sources and, like chemical methods, would not require a known-source library. This method is also under development and is very new, so there is little information available about it at present.

## What MST Methods Are Being Used in Virginia?

There are a variety of specific methods from the above categories that are being used in MST research and in Virginia's Total Maximum Daily Load (TMDL) program. These include the biochemical method of Antibiotic Resistance Analysis (ARA), the molecular method of pulsed-field gel electrophoresis (PFGE), the molecular method of polymerase chain reaction (PCR), and the chemical method of detecting optical brighteners in detergents (fluorometry). Two of these, ARA and PFGE, are described in more detail as they have been relied upon more heavily by the Virginia Department of Environmental Quality (DEQ).

### Antibiotic Resistance Analysis

ARA is currently the most widely used MST method throughout the country. The DEQ has endorsed the use of ARA within Virginia and is seeking to expand and improve this method through MST research. Not only is ARA fairly inexpensive, but it is also quick and reasonably accurate. This method examines bacterial antibiotic resistance, as was previously discussed in the biochemical section. Virginia Tech is involved in ARA related research designed to increase accuracy, to develop larger libraries, and to improve the statistical interpretation of the results. In one method comparison study, the accuracy of ARA (and most other methods in the study) was reported as 30 percent to 60 percent. This range is low when compared to the 60 percent to 80 percent accuracy reported for ARA by other researchers. Such variability reinforces the need for those using MST to be thoroughly aware of the strengths and weaknesses of each method and take care when interpreting the results of a particular method.

### Pulsed-Field Gel Electrophoresis

PFGE is a proven method (developed by the Centers for Disease Control in Atlanta) that uses DNA, as described in the above molecular section, to accurately identify individual sources of fecal bacteria. It is currently being used in Virginia to cross-validate ARA results. Though PFGE is considered more accurate, it is also expensive, time consuming, and difficult to perform. It requires specialized lab personnel to determine results, another reason why it is not widely used. It is typically only used on a small number of samples to reduce costs or, as mentioned before, to cross check the results of other methods. In one method comparison study, the accuracy of PFGE (and most other methods in the study) was reported as 40 percent to 80 percent. This range is low when compared to the 80 percent to 90 percent accuracy reported by other researchers. Once again, such variability reinforces the need for those using MST to be thoroughly aware of the strengths and weaknesses of each method and use caution when interpreting the results of a particular method.

## How Is MST Used in the TMDL Process?

Once a body of water has been listed as impaired by fecal bacteria by the DEQ, a TMDL study must be conducted to determine how the impairment can be remedied so the water body will meet appropriate water-quality standards. Part of each bacterial TMDL study involves a pollutant source characterization that consists of estimating the various bacterial sources and their respective contributions to the water body. Typically, when developing a bacterial impairment TMDL in Virginia, source characterization is accomplished using multiple approaches, including gathering information from state agencies, contacting local *stakeholders*, making watershed reconnaissance visits, finding information published in technical literature, and using the best professional judgment.

MST is another tool that can be used to assist and refine the source characterization processes. MST is a useful tool that can assist TMDL developers, but additional research is needed to improve the accuracy and utility of this tool. While MST is able to differentiate between most sources of fecal pollution, using MST alone to develop TMDLs is not practical. The sources identified in any particular water sample are highly dependent on when and where that sample was collected. So, though MST is a valuable technology, even at this early stage, it is not meant to be used instead of the current TMDL study process that involves using *watershed models* to simulate a range of climate and flow conditions over time to develop the TMDL.

## The Future of MST

MST researchers are working towards developing combinations of methods that are relatively fast, reasonably priced, and do not require host-origin libraries. However, it has been estimated that the availability of such methodology is still some years away. Until that time, the library-based methods described above and currently in use in Virginia (and most other states) will continue to be the best available approach to performing source tracking as a useful component of the overall TMDL program.

## World Wide Web Resources

EPA: Total Maximum Daily Load Program Description

*http://www.epa.gov/OWOW/tmdl/*

Virginia Department of Environmental Quality TMDL Homepage

*http://www.deq.virginia.gov/tmdl/*

Virginia Department of Conservation and Recreation – Soil and Water Conservation

*http://www.dcr.virginia.gov/sw/*

USDA Cooperative States Research, Education, and Extension – Mid-Atlantic Regional Water Quality Program

*http://www.mawaterquality.org/*

**stakeholder** - (in this context) any person or organization with a vested interest in TMDL development and implementation in a specific watershed.

**watershed model** - a computer program that allows the user to simulate specific hydrologic and water-quality processes and conditions. Hydrologic and water-quality models relate “inputs” like watershed characteristics (land use, topography, soil type, and pollutant sources) to “outputs” like runoff and in-stream pollutant levels.

## Companion Virginia Cooperative Extension Publications

*A Glossary of Water-Related Terms.* Virginia Cooperative Extension publication 442-758, *http://pubs.ext.vt.edu/442-758/*

*TMDLs (Total Maximum Daily Loads) - Terms and Definitions.* Virginia Cooperative Extension publication 442-550, *http://pubs.ext.vt.edu/442-550/*

*TMDLs (Total Maximum Daily Loads) for Benthic Impairments.* Virginia Cooperative Extension publication 442-556, *http://pubs.ext.vt.edu/442-556/*

For a complete listing of Virginia Cooperative Extension fact sheets and bulletins, please go to *http://www.ext.vt.edu/resources.*

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