

Title: Comparison of Seven Protocols for Microbial Source Tracking in Berkeley County, West Virginia

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Introduction and Problem

Information is needed to identify the source(s) of the fecal bacteria in ground water (a drinking water source) of Berkeley County. Bacterial source tracking is a new suite of methodologies under development to evaluate probable sources of water-isolated fecal bacteria from streams, rivers, and aquifers (Carson and others, 2001; Dombeck and others 2000; Hagedorn and others 1999; Parveen and others, 1999; and Wiggins and others, 1999). From prior studies, bacterial source tracking appears able to associate fecal-indicator bacteria with animal sources. University researchers and others are currently developing several methods of bacterial source tracking. Some of these methods have been deployed, apparently successfully, to evaluate sources of fecal bacteria to surface water such as streams in small watersheds. Rarely have bacterial source tracking methods been applied to ground water.

Goals and Objectives

The first objective (Phase I) of this study was to compare the ability of seven protocols for bacteria source tracking to associate fecal-bacteria isolates with nine bacteria sources (humans and eight other animal types that are abundant in Berkeley County).

- Reproducibility of associations
- Accuracy of associations
- Sensitivity to previously-unseen sources

The second objective (Phase II) of the study was to associate fecal bacteria isolated from well and spring water in Berkeley County with human and other animal sources. An appropriate source-tracking method identified by the Phase I evaluation was to be used during Phase II to evaluate sources of fecal bacteria to contaminated water. No appropriate protocol was found in Phase I; thus, action on Phase II was suspended.

Approach

Five bacteria source tracking methods, two by competing protocols (total seven protocols), were evaluated in phase 1 of this study beginning FY01. The five methods selected were ribotyping (Carson and others, 2001), pulsed field gel electrophoresis, antibiotic resistance analysis (Hagedorn and others, 1999), rep-PCR (Dombeck and others, 2000), and carbon source utilization. Ribotyping, pulsed field gel electrophoresis, and rep-PCR are all examples of molecular methods, collectively referred to as "DNA fingerprinting". Antibiotic resistance analysis and carbon-source utilization profiling are biochemical methods. Each method used *Escherichia coli*, a regulatory fecal-indicator bacterium, to accomplish source tracking.

Source tracking using fecal-indicator bacteria is achieved by comparing water-isolated *E. coli* with known-source *E. coli*. About 20 feces samples were collected from each source group (humans, beef cattle, dairy cattle, poultry, horses, swine, dogs, white-tailed deer, and Canada geese). Feces were cultivated by the USGS/WRD/Ohio District microbiology. Identical libraries of confirmed *E. coli* were sent to the participating laboratories - antibiotic resistance analysis by Bruce Wiggins; the REP-PCR variant of rep-PCR by Don Stoeckel; the BOX-PCR variant by Howard Kator; the HindIII variant of ribotyping by George Lukasik; the EcoRI/PvuII variant of ribotyping by Mansour Samadpour; pulsed-field gel electrophoresis by Kriston Strickler and Tara O'Brien; and carbon-source utilization profiling by Charles Hagedorn.

The source libraries for each of the seven bacteria source tracking protocols consisted of 70 or 100 isolates for each of the nine potential sources (3 to 5 isolates from each of the nominally 20 feces samples, depending on requirements of the protocol). After the source libraries had been analyzed by each method, a challenge set of isolates was assembled. The challenge set consisted of 200 isolates:

1. 25 isolates re-cultivated from the original known-source library -- reproducibility
2. 150 isolates as 1 *E. coli* isolate from each of nominally 15 new feces samples per source (30 from humans) -- accuracy
3. 25 new feces samples from previously unseen sources such as llamas, cats, and raccoons -- sensitivity.

Each participating laboratory attempted to identify the source of each challenge isolate to an individual source (9-way split) or to a group of sources (3-way split: human, domestic, wild; 2-way split: human, non-human). Associations of the challenge isolates were reported to Melvin Mathes and scored as correct or incorrect. The results have been summarized in a manuscript and submitted for consideration by Environmental Science and Technology. If accepted, the paper should be published around Spring 2004 as "Comparison of Seven Protocols to Identify Fecal Contamination Sources using *Escherichia coli*: Berkeley County, West Virginia" by Donald M. Stoeckel, Melvin V. Mathes, Kenneth E. Hyer, Charles Hagedorn, Howard Kator, Jerzy Lukasik, Tara L. O'Brien, Terry W. Fenger, Mansour Samadpour, Kriston M. Strickler, and Bruce A. Wiggins