

Comparison of Seven Protocols To Identify Fecal Contamination Sources Using *Escherichia coli*

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Abstract:

Microbial source tracking (MST) uses various approaches to classify fecal-indicator microorganisms to source hosts. Reproducibility, accuracy, and robustness of seven phenotypic and genotypic MST protocols were evaluated by use of *Escherichia coli* from an eight-host library of known-source isolates and a separate, blinded challenge library. In reproducibility tests, measuring each protocol's ability to reclassify blinded replicates, only one (pulsed-field gel electrophoresis; PFGE) correctly classified all test replicates to host species; three protocols classified 48-62% correctly, and the remaining three classified fewer than 25% correctly. In accuracy tests, measuring each protocol's ability to correctly classify new isolates, ribotyping with *EcoRI* and *PvuII* approached 100% correct classification but only 6% of isolates were classified; four of the other six protocols (antibiotic resistance analysis, PFGE, and two repetitive-element PCR protocols) achieved better than random accuracy rates when 30-100% of challenge isolates were classified. In robustness tests, measuring each protocol's ability to recognize isolates from nonlibrary hosts, three protocols correctly classified 33-100% of isolates as "unknown origin," whereas four protocols classified all isolates to a source category. A

relevance test, summarizing interpretations for a hypothetical water sample containing 30 challenge isolates, indicated that false-positive classifications would hinder interpretations for most protocols. Study results indicate that more representation in known-source libraries and better classification accuracy would be needed before field application. Thorough reliability assessment of classification results is crucial before and during application of MST protocols.