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DETECTING MULTIPLE SOURCE ORIGINS FROM FECAL BACTERIA ANTIBIOTIC RESISTANCE PATTERNS

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Fecal bacteria in surface waters are one of the most commonly cited impairments to water quality. These impairments are generally attributed to malfunctioning septic systems, wildlife defecation in water, and land-applied wastes. Given the nature of contaminants, this study was initiated to evaluate microbial source tracking (MST) as a management tool to differentiate nonpoint source pollution into source groups.

Microbial source tracking (MST) techniques offer unique methods for differentiating nonpoint source pollution. By tracking a pollution source to its origin, resources and management tools may be better allocated to improve water quality. Many of these techniques require a database or 'library' for identifying the host origin; however, appropriate database size, portability, and temporal characteristics to yield adequate power of prediction given the diversity of patterns in a watershed are as yet undetermined. Use of phenotype-based MST methods, such as antibiotic resistance analysis (ARA), has shown great potential for characterizing nonpoint source pollution. Simpson et al. (2002) reported on the practicality of using the ARA technique for small watersheds due to its relative simplicity and expense; however, there is no consensus on the optimum database size for representativeness of a given area, nor is there a consensus on whether using either *Escherichia coli* or *fecal streptococci* as indicator bacteria is preferred. Merely increasing database size does not necessarily correspond to an increase in rates of correct classification (RCC). There are more complex factors involved such as genetic diversity of the bacteria (i.e., clonal nature), geographical area, antibiotic array, source character, and temporal characteristics.

Given the labor-intensive and time-consuming nature of database building, the characteristics of a useful database are critical in the future applicability of MST methodologies. An existing database originally containing poultry, human, and wildlife sources of *E. coli* (EC) and fecal streptococci (FS) was enlarged by three additional host groups - horse, dairy cattle, and beef cattle - to better represent the central Kentucky area. Sample material was collected from manure, soil, sod, water, and sediment. The samples were enumerated for FC and FS and representative EC and FS isolates were selected. Antibiotic resistance analysis (ARA) was conducted on these isolates using a profile of seven antibiotics: ampicillin (10 µg), cephalothin (30 µg), erythromycin (15 µg), rifampin (5 µg), streptomycin (10 µg), tetracycline (30 µg), and trimethoprim (5 µg).

The rates and average rate of correct classification (RCC and ARCC, respectively) of the database were all below a 60% threshold criterion when analyzed at the species-level (Fig. 1). The domestic animal source RCC was 63% for EC and 69% for FS and the ARCC was 57% for EC and 65% for FS with the human and wildlife groups below the threshold when analyzed at the management-level (Fig. 2). All groups were above the threshold level when analyzed at the human/nonhuman-level of classification (Fig. 3). The RCC for human source groups was 66% for EC and 75% for FS. The RCC for nonhuman source groups was 67% for EC and 70% for FS. The ARCC of the database was 67% for EC and 74% for FS.
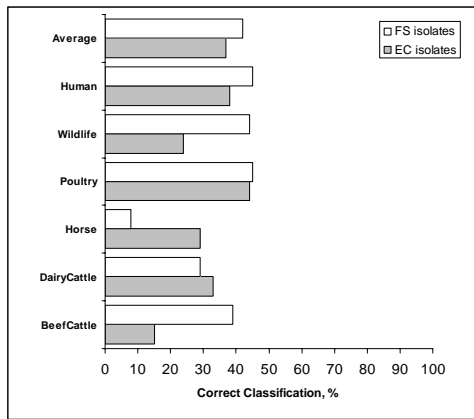

Figure 1. Species-level classification (six-way split) of *Escherichia coli* (EC) and fecal streptococci (FS) known source databases based on rates of correct classification using resubstitution.
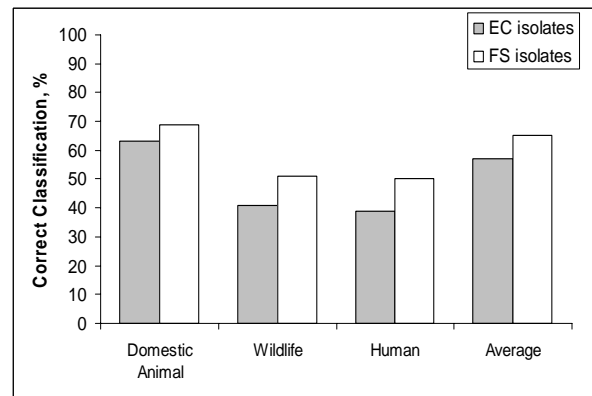

Figure 2. Management-level classification (three-way split) of *Escherichia coli* (EC) and fecal streptococci (FS) known source databases based on rates of correct classification.

The database used in this study was sufficiently useful, despite its small size, to be a resource management tool to delineate human verses nonhuman sources of nonpoint pollution regardless of indicator bacteria used, i.e. EC or FS. The application of MST techniques as resource management tools may be optimally applied when used for confined geographical regions. This could particularly hold true when the various inputs of fecal contamination are expected to be relatively continuous.
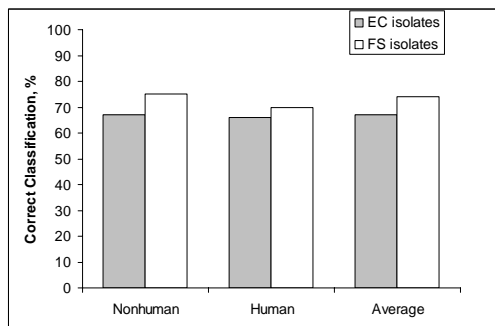

Figure 3. Human/nonhuman-level classification (two-way split) of *Escherichia coli* (EC) and fecal streptococci (FS) known source databases based on rates of correct classification using resubstitution.

References

Simpson, J.M., J.W. Santo Domingo, and D.J. Reasoner. 2002. Microbial source tracking: State of the science. Environ. Sci. Technology 36:5279-5288.

MICROBIAL SOURCE TRACKING: LIMITS AND APPLICATION

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Waterborne disease has long been a public health issue. According to one recent report, more than five million people die each year from water-related disease and the leading cause of child death worldwide is diarrhea. The United States Environmental Protection Agency lists pathogen (or microbial) pollution as the second most common cause of impairment in watersheds throughout the US. In Kentucky, the US EPA's 1998 303(d) list indicates that 20,964 acres of wetland and lake as well as 1,289 miles of stream and river exceed pathogen standards. Thus, controlling microbial (or fecal) pollution is a local issue as well as a national and international one.

To date, methods for remediating microbial pollution have been generally unreliable. Traditionally, the answer has been to monitor concentrations of fecal indicator organisms, like total coliform and E. coli. *However, simply monitoring the organisms doesn't identify the source and the potential for disease outbreak and environmental impact.*

More recently, researches have begun to focus on methods to identify the source of the contamination. Microbial source tracking (MST) techniques enable researchers to identify the dominant source of microbial pollution, especially on smaller scale watersheds. Currently, there are several methods available offering differing degrees of source discrimination and reliability. All source tracking tools operate on the assumptions that the bacteria or viruses of interest are geographical and temporally stable, host specific, and found representatively in water or soil sampling.

Comparative studies reveal that all MST methodologies have not developed to the same degree, nor do they meet identical objectives. These studies tested the successfulness of differing methodologies to correctly classify blind samples containing human, dog, cattle, cow, gull, or sewage sources of microbial contamination. Results indicate that no methodology can correctly classify all blind samples correctly, but depending upon the objective of the project, can be applied successfully. For example, library-based methods, to differing degrees, can correctly identify the dominant source, but have high rates of false positives and require the development of large isolate libraries. Library-independent methods are the most accurate and cost efficient, achieving up to 92% correct identification of human and cattle, but have not yet been developed to identify all host-specific sources.

MST methods should be applied in a toolbox fashion selecting the appropriate tools based upon the individual watershed and project objectives. Before MST analysis is conducted, watershed size, number of potential sources, resolution required between those sources, time and financial constraints, and other relevant factors must be taken into consideration. Based upon the evolving state of the science, understanding the wide array of methodologies (*with variable assumptions and performance*) is essential to targeting successful remediation strategies.

TRIPLETT CREEK FOCUS STUDY

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Triplett Creek is a fourth-order Licking River tributary that flows through the city of Morehead, Kentucky in Rowan County. Like many eastern Kentucky rivers and streams, it is impacted by point and non-point pollution, especially contamination from sediment and potentially pathogenic microorganisms. This pollution could have it origins in agricultural, urban, and suburban non-point runoff. Many regional residences and businesses lack adequate waste-water treatment; however, compared to other eastern Kentucky regions, Morehead has a greater percentage of residents and businesses that utilize city sewer. We analyzed water quality in Triplett Creek and its major tributaries from June through September 2006 to identify which tributaries and regional landscape uses had the greatest impact on water quality in Triplett Creek. We measured stream biological components (fecal coliform, fecal streptococcus, and E. coli bacteria) and physiochemistry (flow and discharge, dissolved oxygen, pH, temperature, nitrate, nitrite, ammonium, total suspended solids, conductivity, alkalinity, sulfate, and iron). All measured streams showed some degradation; however, some tributaries were of particular interest because they exceed limits for use by humans or aquatic life.

NOTES