

# Microbial Source Tracking

Katharine G. Field<sup>1</sup> and Troy M. Scott<sup>2</sup>

<sup>1</sup>Associate Professor and Co-Director, Bioresource Research Interdisciplinary Program,  
Department of Microbiology, Oregon State University, Corvallis, OR 97331

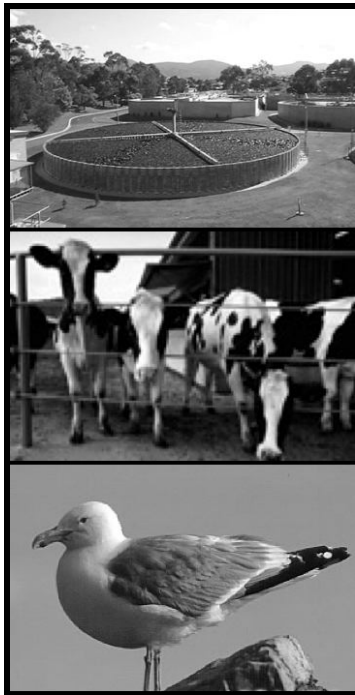
([kate.field@oregonstate.edu](mailto:kate.field@oregonstate.edu))

<sup>2</sup>Scientific Director, Source Molecular Corporation, 4989 SW 74th Court, Miami, FL 33155

([tscott@sourcemolecular.com](mailto:tscott@sourcemolecular.com))

## Introduction

Microbial contamination affects a large number of water bodies in the US. Because it is not feasible to directly monitor all pathogens, it is standard practice to monitor fecal indicator bacteria (FIB), such as total and fecal coliforms, *Clostridium perfringens*, *Escherichia coli*, and fecal enterococci, in water. Epidemiological studies have established human health standards based on exposure to FIB in drinking, recreational and shellfish waters. Most US epidemiological studies, however, took place at sites where the principal source of fecal contamination was human sewage, because human fecal contamination is usually assumed to present a more serious threat to health than contamination from animals.



The health threat from human fecal contamination is well documented. Domestic/agricultural animals also spread many pathogens; for example, *Salmonella*, *E. coli* O157:H7, *Campylobacter jejuni*, *Giardia spp.*, *Cryptosporidium spp.*, and hepatitis E virus. The human risk from domestic/agricultural animal feces is usually assumed to be less than from human feces, in part because viruses, a common cause of illnesses from exposure to feces, are highly host-specific. However, few studies have actually measured this risk. In a Hong Kong study, rates of GI tract illnesses for two marine beaches impacted by animal (pig) wastes were lower than for seven other beaches, although rates of skin illnesses were higher (31, 74). In a New Zealand study carried out at marine beaches, no substantial differences in illness risks were found between human and animal (“rural”) waste impacted beaches, although both were greater than control beaches (106).

The disease risk from fecal contamination of wild animals is poorly understood, although a number of emerging and re-emerging waterborne zoonotic pathogens have been recognized (12). Certain waterborne bacterial and protozoan

pathogens of wild animals infect humans (e.g. *Leptospira interrogans*, *Salmonella*). *Giardia* and *Cryptosporidium* widely infect wild animals. However, molecular evidence indicates that most genotypes of these parasites are host-adapted and cannot cross-infect among different host species (4, 174, 175). A recent exposure study at Mission Bay, California (34) found a much lower level of human illnesses than expected considering the levels of FIB. In the next year, a follow-up study used two different methods of microbial source tracking (ribotyping, *Bacteroidales* markers) and found that the primary source of the FIB at Mission Bay was non-human, most likely from water birds (M. Samadpour and K. G. Field, unpublished data).

Humans and animal species contain both different absolute numbers and different ratios of *E. coli* and enterococci in their feces, although data are contradictory (2, 47, 50, 57, 167). This makes it unclear how to estimate the *E. coli* or FIB contribution of different sources of feces when sources are mixed.

The use of FIB has significant drawbacks, including the ability of FIB to reproduce in the environment (19-22, 53, 78, 120, 168, 169) and the poor correlation of FIB with many pathogens (13, 56, 69, 75, 81, 96, 101, 116, 131). One of the most important limitations is that indicator bacteria don't identify the source of the contamination. A variety of warm- and cold-blooded animals contain FIB in their feces (60, 68, 152). This has motivated the emerging field of microbial source tracking.

### **Microbial Source Tracking**

Microbial source tracking (MST) is one of several names given to the process of identifying the particular source (e.g., human, cattle, bird) of fecal contamination in water. The underlying assumption of microbial source tracking is that some characteristic associated with feces from a particular source ("host species") allows that type of feces to be identified. This characteristic must be detectable in water. Some methods make the (usually untested) assumption that the relative proportion of such identifying traits remains the same over time, allowing source tracking to be quantitative. There are several good reviews of microbial source tracking (47, 110, 139, 144, 148, 150, 173).

Although there are many proposed methods of microbial source tracking, only a few have been given rigorous testing, such comparison to other methods using blind samples. There are even fewer published studies in which a source tracking method has been used to identify sources, and a resulting improvement in water quality has been measured. Here we review better-known methods of microbial source tracking, give examples of case studies, and formulate some general recommendations. We use the term "host-specific marker" to mean any trait used to identify a fecal source to its host or source species, including chemical markers, fingerprints, and DNA sequence targets for host-specific PCR.

### **Methods of Microbial Source Tracking**

Methods for fecal source identification can be divided into culture-based and culture-independent methods. Some methods require a "library" (also called a "host origin database"), a set of bacterial isolates or patterns from fecal samples of known origin, tested using the method of source discrimination. Most library methods are culture-based, and require growing environmental isolates from water samples. Source identification occurs by a comparison between test patterns from the library and the environmental isolates. Library-dependent methods include both phenotypic and genotypic tests. Culture-dependent, library-independent methods are based on growing source-specific viruses or bacteria. Library-independent, culture-independent methods include chemical and molecular tests.

**Culture-based, library-dependent methods.** Library-based methods typically use *E. coli* or enterococci.

**Phenotypic methods.** Prominent phenotypic methods include antibiotic resistance analysis (ARA, MAR, ARP), carbon-source utilization profiling, serotyping, and fatty acid methyl ester (FAME) profiling. In antibiotic resistance methods, isolates of *E. coli* or enterococci are tested against panels of antibiotics in order to discriminate human and various animal sources of fecal pollution (24, 42, 65, 70, 71, 124, 171). The underlying assumption is that humans, agricultural animals, and wildlife have been exposed to different antibiotic regimes, and therefore their fecal

bacteria will differ in types and levels of antibiotic resistance. There are numerous reasons why this assumption doesn't necessarily hold, including lability of plasmid-borne resistance and environmental exposure to antibiotics. Comparative studies assessing antibiotic resistance methods for fecal source tracking have given them low ratings (71, 111, 136). In a blind study that compared a number of fecal source tracking methods using water samples containing feces, antibiotic resistance-based methods performed poorly (63).

In carbon-source utilization profiling (CUP), also called biochemical or phenotypic fingerprinting, commercially available microplate systems containing substrates for bacterial growth (e.g. Biolog, PhPlate) are used to generate patterns of substrate utilization from fecal and water isolates. CUP performed poorly at identifying blind samples in a comparative study (63, 71).

Because of the requirement for many antisera, the serotyping approach has not been pursued past an initial proof-of-concept study (123). Fatty acid methyl ester (FAME) profiling for source tracking is at an early stage of testing (39, 73, 123, 143). Serotyping and FAME have not been compared with other methods, nor have they been tested using blind samples.

**Genotypic methods.** Genotypic library-based methods are usually based on DNA fingerprinting of bacterial isolates, producing bar code-like patterns for each isolate. Fingerprints from individual water isolates are matched to fingerprints of isolates from known sources of feces (the library). Techniques include ribotyping (26, 27, 66, 112, 125, 135), repetitive extragenic palindromic polymerase chain reaction (REP-PCR) with REP, BOX or ERIC primers (27, 38, 77, 84, 108), amplified fragment length polymorphism (AFLP) (64, 97), pulsed-field gel electrophoresis (PFGE) (112), random amplified DNA polymorphisms (RAPD) (165), and denaturing gradient gel electrophoresis (DGGE) (147). Currently there are two different ribotyping protocols in common use. The protocol developed by Samadpour (63, 112, 135) uses two restriction enzymes (EcoR1 and PvuII), while other investigators have used a HindIII based ribotyping protocol (26, 111, 125, 141).

Ribotyping, PFGE and REP-PCR have progressed to the methods comparison stage of testing; the other fingerprinting methods have not. In a blind study that compared a number of fecal source tracking methods using water samples containing feces (see below), the performance of ribotyping (with the Samadpour protocol) and PFGE (using XbaI) was good (63, 112); REP-PCR was not as good. Success of methods varied in the hands of different investigators.

For fingerprint methods, the size of the "library" is extremely important, as is the method of analysis (1, 72, 77, 84, 97, 155). In addition, depending on the size of the library, many or most environmental isolates cannot be matched to fecal isolates (72, 155). It is necessary to discard these unmatched isolates; identifying them based on similarity to, rather than identity with, known isolates results in incorrect classifications. This observation fits with evidence that environmentally-adapted, rather than fecal, genotypes of FIB dominate extra-intestinal habitats (87, 107, 128). Library-independent, culture-dependent methods.

**Bacteriophage methods.** Phage methods are currently limited to discriminating between human and non-human sources. Bacteriophages from *Bacteroides fragilis* can discriminate human and non-human feces (130, 160, 161). However, these phages are not common in the US and Canada (reviewed in (126)). Similarly, two serotypes of F+ RNA coliphages, Types II and III, are found in human feces, whereas Type IV is found in animal feces and Type I occurs in both human and animal feces (54, 67). Growth of these coliphages in cell culture, followed by serotyping, identifies human and non-human fecal contamination (33, 154).

Phage types exhibit differential survival (100) and are irregularly distributed in populations, working better in some geographic areas and when fecal sources comprise multiple individuals

(such as sewage) rather than single individuals (115, 126). Phage methods were successful at identifying human sewage in blind samples in a comparative study (115).

**Bacterial methods.** An earlier method to distinguish human from non-human fecal contamination was based on the ratio of fecal streptococci to fecal coliforms; this ratio differs in fresh feces from humans and agricultural animals (58). But because coliforms and streptococci have different survival rates, the ratio changes in complex ways over time, making it unreliable (149, 150).

Several microbial source tracking methods are based on culturing host-specific bacterial strains, such as *Bifidobacterium adolescentis* and sorbitol-fermenting bifidobacteria for humans (14, 102, 104, 105, 132), and *Rhodococcus coprophilus* for grazing animals (104, 105, 121, 134, 137). They are isolated with selective media and detected with colony hybridization; molecular detection is coming into widespread use. Environmental survival of bifidobacteria is brief and they cannot be detected at higher (summer) temperatures (14, 23, 132, 133). These methods have not been tested with blind samples.

### **Culture-independent, library-independent methods**

**Chemical methods.** Caffeine, fecal sterols and stanols, bile acids, laundry brighteners, surfactants including linear alkyl benzenes, fragrances, pesticides, and polycyclic aromatic hydrocarbons can all be used to detect human and non-human fecal contamination or determine urban and agricultural runoff (16-18, 43, 79, 95, 114, 129, 150, 153, 157, 159). In samples from a broad geographic region, European investigators found that the relative amounts of two sterols, 24-ethylcoprostanol and coprostanol, distinguished human and non-human samples (10).

These methods are widely used in certain parts of the world, including Europe and Australia, but have not been compared to other methods or tested with blind samples. Drawbacks of chemical indicators and molecular tracers are that their spread, transport, and persistence in water may not be correlated with that of pathogens and FIB. Some are removed by wastewater treatment (for example, caffeine), while others persist in sediments, often for years, and may be resuspended later (5, 16, 40, 44, 45).

**Molecular methods.** In these methods, a genetic marker is assayed directly from a water sample or from DNA extracted from a water sample, without an intervening culture step. Marker genes are assayed by the polymerase chain reaction (PCR), a method of making millions of copies of a specified DNA sequence in a test tube. For that reason this approach is also called "host-specific PCR." The approach speeds up the process of source tracking, and allows access to novel markers that would be difficult or impossible to get by culturing. These methods can theoretically take as little as two or three hours from sampling to diagnosis.

**Viral methods.** Host-specific fecal viruses, including human adenoviruses, enteroviruses, and polyoma virus; porcine teschoviruses and adenoviruses; and bovine/ovine adenoviruses and enteroviruses (52, 62, 76, 81-83, 98, 103, 109, 116, 127, 163) can be monitored directly in water, without culturing (reviewed in (51, 61)). The human and porcine viruses appear to be highly host-specific; they have been monitored in surface waters in the US and Europe. In the US and Spain, bovine enteroviruses are not species-specific, occurring in cattle, deer, sheep, goats, horses, and geese.

A drawback is that monitoring for viruses may require water samples larger than 100 ml samples used for standard FIB monitoring. Concentration of large samples can concentrate PCR-inhibitory substances as well, interfering with detection (81, 156, 158).

Viral methods performed well in detecting human sewage in a comparative source-tracking study, although they often missed individual human fecal samples (115). These methods are particularly important because they may directly detect viral pathogens, which are not well correlated with FIB, thus giving some information on pathogen status that is not provided by indicator counts.

*Anaerobic bacteria: Bacteroidales and bifidobacteria.* Many fecal anaerobic bacteria have host-specific distributions (3, 49, 88, 132). The majority of bacteria in feces are fecal anaerobes, present at much higher densities than coliforms and enterococci. These anaerobes were not generally used as indicators until molecular detection was adopted. "Uncultivated" fecal anaerobic bacteria (strains or species that have never been grown in the lab) are a good source of host-specific molecular markers, and are more common in feces than cultivated ones (41). Ribosomal RNA genes, present in multiple copies in most bacteria, are often used as targets, increasing the ease of detection. Host-specific protein gene targets from fecal anaerobes have also been identified (146).

*Bacteroides* and related genera in the order *Bacteroidales* are common in feces, making them relatively easy to detect. They are genetically diverse, are limited to animal body cavities, have very limited reproduction after release into receiving waters (because they are anaerobes), and show host species- or group-specific distributions (3, 49, 88). *Bacteroidales* host specific PCR primers based on uncultivated microbes can identify feces from ruminants, cows, humans, dogs, pigs, horses and elk (6, 35, 37). A PCR assay for *B. thetaiotaomicron* distinguishes human and dog feces from other animals (25).

In 100 ml natural water samples, the limit of detection of *Bacteroidales* host-specific markers is comparable to the limit of detection of *E. coli* by culture (6, 8, 15, 36). *Bacteroidales* assays appear to be extremely geographically stable, and have been used throughout the United States and Canada, northern Europe, Hawaii, Japan and New Zealand ((9, 11, 15, 59, 89, 92, 119, 140, 142, 145, 151, 166). *Bacteroidales* markers also correlate with sewage and FIB (36, 93), and some are good predictors of the occurrence of zoonotic pathogens (166). In a blind comparative study of fecal source tracking methods, host-specific PCR of *Bacteroidales* molecular markers performed well, giving 100% correct classification with no false positives or negatives (48, 63).

Limitations of this approach include a lack of markers for many species, especially wildlife. In addition, horizontal transfer of fecal bacteria among species in close contact is well known (e.g. humans and their pets; (28, 35, 170)). Because molecular markers potentially can move from one species to another, specificity must be tested whenever *Bacteroidales* PCR markers are used.

Similar approaches have targeted the genus *Bifidobacterium* (113). However, there have been problems both with host specificity, and with poor detection due to low survival in water (7, 23, 132). Source tracking using *Bifidobacterium* has not been tested against blind samples.

*Toxin/virulence genes from FIB.* PCR assays for fecal source tracking have targeted toxin genes in *E. coli* and enterococci. Advantages include host specificity and presumed geographic stability (since the toxin-containing strains are known to occur worldwide).

Targets from *E. coli* include the human-specific STIb toxin (122), the pig-specific STIb toxin (86), and the cattle-specific LTIIa toxin (30, 85).

An assay for a human variant (94) of a virulence factor from *Ent. faecium*, the enterococcal surface protein (*esp*) indicates human contamination (140). In a US test, 97% of human sewage and septage samples, but no livestock or bird samples, were positive for this *esp* marker (140).

A drawback of assays that detect virulence genes is that the target genes are rare. From a 100 ml water sample, the target must be increased by either enriching *E. coli* or enterococci, or performing nested PCR or magnetic bead capture (162), before the toxin genes can be detected. Detection is thus semi quantitative. If enrichment is used, the method is not culture-independent, and will take longer than methods that directly sample genes without an intervening growth step. However, the method should work without enrichment in larger water samples. A second potential drawback is instability due to horizontal transfer of genes or cells, a well-known process in pathogenicity genes (e.g. see (32, 118, 164).

In a blind study that compared a number of fecal source tracking methods, host-specific PCR of *E. coli* toxin genes performed well (48). However, a human *E. coli* toxin gene marker occurred in a non-human fecal sample, possibly due to horizontal transfer (48). Since the *Enterococcus esp* method is recent, it has not been compared to other methods using known-source samples, or assessed for its ability to identify blind samples, although it has been applied in field studies in Michigan and Florida (80, 99, 109).

### **Culture-independent Versus Culture-Dependent Methods**

**Advantages and limitations of culture-based methods.** Culturing fecal indicator organisms is relatively inexpensive and low-tech, making it broadly available. However, this initial advantage may be lost if the source identification method that is applied to the cultured isolates is high-tech and expensive (e.g., PFGE). Another advantage of culturing is that it provides an enrichment step, increasing the numbers of target microorganisms and/or providing single strains in isolation. The down side of this is that after enrichment, detection can only be semi-quantitative. Culture-based methods often use standard public health indicators such as *E. coli* or enterococci, for which some information about survival, transport and correlation with pathogens and disease is already available.

Disadvantages are that the composition of microbial communities changes drastically when cultured (46). This “culture bias” is rarely considered in culture-based fecal source identification, and has important implications for attempts to use these methods quantitatively. Also, library-based approaches are labor-intensive, requiring extensive sampling both to prepare the library and to test environmental isolates. They have complex requirements for adequate sample size, representativeness, and geographic stability (71, 84, 112, 172). For some methods, libraries are not cosmopolitan, and thus a separate library for each locale or watershed may be required (e.g., see (66, 123, 141); however, these studies were based on libraries of limited size).

**Advantages and limitations of culture-independent molecular methods.** These methods have the advantage of sampling the entire population present in the sample, with no culture bias. In addition, they are simpler and quicker than culture-based methods; they may require only a few hours to detect fecal pollution and identify its source. They do not require prior preparation of a “library,” as the markers are in most cases universal or nearly so. They are not limited to easy-to-culture microbes, but can instead use difficult-to-grow but common fecal microbes or mine the uncultivated genetic diversity in feces for markers.

However, host-specific markers may not be present in every individual of a species, and individuals may have differing amounts of the markers. As a result, these methods often work better to detect “bulk” or community samples (such as sewage, for humans) rather than samples

from single individuals. Also, some markers may be small or rare (viruses, toxin genes), potentially requiring large water samples or an enrichment step to detect them.

A drawback of using any markers other than FIB is that their survival relative to, and correlation with, standard fecal indicators and pathogens are poorly known. Since regulations are currently based on FIB, alternative markers must be correlated with public health bacteria in order for them to be most useful. A further limitation of the culture-independent methods is that markers for only a few animal species are currently available; wildlife species especially are poorly represented. More and different gene targets are needed.

### **Comparative Studies**

The Southern California Coastal Water Research Project (SCCWRP) and the US EPA sponsored a study comparing source tracking methods using blind samples (48, 63, 71, 112, 115). Study participants were provided with identical sets of water samples containing human, cattle, dog, or gull feces, sewage, or a mixture, and asked to identify the fecal source(s). Participants were also supplied with samples of the feces used to create the blind samples. Methods were assessed according to their ability to identify whether samples did or did not contain human feces, identify each fecal source, quantify fecal contributions, and handle freshwater and saltwater samples and samples with humic acids.

Host-specific PCR (of *E. coli* toxin genes and *Bacteroidales* markers), ribotyping (Samadpour protocol) and PFGE (Samadpour protocol) performed the best. Several of the other methods identified most or all samples with human input, but had very significant numbers of false positives. The virus-based methods worked well at identifying samples with sewage but less well at identifying samples with human feces. None of the methods correctly identified all the sources in every sample. The same approach did not perform equally well in the hands of different investigators, underlining the need for standardization. The rate of false positives for some culture-based, library-dependent methods was very high. No method accurately quantified the sources (63).

Smaller comparative studies of library-dependent methods alone (111, 136, 155) have found ribotyping (Samadpour protocol) and PFGE worked well, while ribotyping (single enzyme protocol) and antibiotic resistance analysis did not. Few studies have accurately measured water quality improvements that resulted from source tracking.

However, in the next section, we use case studies to illustrate how microbial source tracking combined with well-planned microbial monitoring plans have provided enough information to facilitate remediation.

### **Examples and Case Studies**

The success (or failure) of a source-tracking project lies in the types of methods employed, the specific approaches taken, and the types of analytical tools used to interpret the results.

- Specific knowledge of the watershed and potential fecal inputs is a critical component of a successful source tracking study. By concentrating only on potential inputs, the methods employed can be specifically targeted to identify these sources.
- All methods should be employed in a tiered fashion and results should be interpreted sequentially so as to implicate or eliminate potential sources one at a time.
- The sampling approach should be well planned and should be conducted during both high flow (wet) and low flow (dry) events. Care should also be taken to perform what is referred to

as “targeted sampling”. Using this approach, samples are taken both upstream and downstream of potential fecal inputs. If bacterial counts are low upstream and high downstream (and source tracking data indicates a strong correlation with the suspected source(s)), then this is a strong indication that the source has been identified. Conversely, this approach can also eliminate suspected sources and redirect attention to other potential impact sites.

- End-users are encouraged to adopt a “toolbox” approach to any source tracking study. By using multiple methods that target and identify different source indicators, more comprehensive conclusions can be drawn. The most important aspect of the toolbox approach is that individual results can be confirmed and substantiated. Conclusions regarding potential inputs should NEVER be drawn due to the results of a single test on a single water sample. On the contrary, multiple positive results using a variety of methods are a strong indicator that the source(s) of the pollution have been identified.
- If budgetary constraints are present, end users are encouraged to conduct their source tracking studies in a piecemeal fashion. It is better to target one or two potential sources with several tests, rather than to try to pinpoint all the potential sources with only one test per suspected source. Most source tracking projects don’t produce meaningful results because too many sources of fecal pollution are targeted simultaneously. By limiting the number of potential targets, the end user can work by a process of elimination towards the likeliest sources of contamination.

### **Case Study #1**

**A utility is concerned about the high levels of fecal indicator organisms being detected in some creeks within the city. It is suspected that faulty infrastructure is to blame and that the utility is responsible for the repair and remediation of this problem.**

**Preliminary investigation:** Initially, the utility identifies the locations of sewer infrastructure in the city. Two sites with unusually high bacterial counts are of particular interest, as they are not located near sewer or outfall lines. These sites are chosen for initial investigation.

**Source tracking:** Because faulty sewer infrastructure is suspected, human fecal pollution is initially investigated. Water samples are screened by polymerase chain reaction (PCR) for the presence of two human fecal molecular markers: a human-specific *Bacteroidales* marker (6) and a toxin gene from human associated enterococci (138).

The results of both tests are strong positives. This is a strong indication of human fecal pollution. To further substantiate the results, an additional test is conducted to detect a molecular marker in a human associated virus. These results are also positive.

Although the type of pollution has been identified, the specific source is still unknown. Targeted sampling upstream and downstream of the sampling site reveals a strong human signal coming from a stormwater runoff drain. This is unusual as there are no combined sewers in the city.

**Result:** Smoke tests conducted by the city reveal a number of residences with sewer/septic systems that are illegally connected to the stormwater drain running near their homes. The connections are repaired and the bacterial levels in the creeks recede.

### **Case Study #2**

**Routine surface water monitoring for *Cryptosporidium* has resulted in several positive results for a watershed in close proximity to a dairy farm (CAFO). Bacteriological tests**

**reveal low to moderate levels of total and fecal coliforms. Runoff from the CAFO is the suspected source of the apparent fecal contamination and *Cryptosporidium*.**

**Preliminary investigation:** The owner/manager of the dairy operation surveys his current manure management system, including the potential of overflow into the adjacent creeks during heavy rainfall. He is unable to identify any potential input sites. The rural farm is the only obvious source of the fecal pollution, however. The farm is also on a septic system.

**Source tracking:** The only apparent sources of fecal pollution in this case are human and cattle. Although cattle pollution is suspected, it is important to initially implicate or rule out a human source of pollution. Water samples are screened for human specific *Bacteroidales* and *Enterococcus* markers and the results are negative. Next, water samples are screened for a ruminant-specific *Bacteroidales* marker and a cow specific *E. coli* toxin gene marker (6, 85). The results of these tests are negative.

Because the presence of *Cryptosporidium* in the watershed was the impetus for this investigation, the area is sampled again. Direct enumeration of *Cryptosporidium* by immunofluorescence microscopy (EPA Method 1623) reveals levels to be 0.08 oocysts/L.

**Additional source tracking:** A duplicate water sample is filtered and processed for *Cryptosporidium* by EPA Method 1623. The concentrated sample is processed for DNA extraction and a *Cryptosporidium*-specific gene is targeted by PCR. Restriction fragment length polymorphism (RFLP) and sequence analysis of the gene reveals the genotype of the *Cryptosporidium* as one found predominantly in birds and nonpathogenic to humans.

**Result:** A survey of the watershed indicates a large migratory bird population. The dairy is no longer suspected as the source of the fecal pollution. *Cryptosporidium* levels decrease to nondetect levels during the summer months. Identification of the source of the *Cryptosporidium* is logged into the State TMDL.

### **Case Study #3**

**A drinking water supply in a small rural community is suspected as the cause of a cluster of individuals presenting with acute gastrointestinal illness (AGI). Bacteriological analyses conducted at the drinking water treatment facility as well as at individual households are negative for total coliforms, fecal coliforms, and enterococci.**

**Preliminary investigation:** The utility reviews bacteriological data for the previous months and identifies no positive results for total coliforms. By showing zero failures for the past 60 days, it concludes it is not the source of the problem.

**Source tracking:** Several water samples are collected from individual households and analyzed for human specific molecular markers by PCR. Human specific *Enterococcus* tests are negative; however, human specific *Bacteroidales* tests are positive. Subsequent tests reveal the presence of a human adenovirus in one of the samples by PCR (29, 55). Chemical analysis of the tap water reveals a chlorine residual of 0.0 ppm.

**Interpretation:** The human specific *Bacteroidales* marker is a culture-independent source tracking method and thus detects both viable and nonviable cells. The chlorine residual of the drinking water when it leaves the plant is sufficient to inactivate 100% of total and fecal coliforms as well as enterococci. Human enteric viruses are more resistant to disinfection by chlorine than bacterial indicators and require longer contact times for inactivation.

**Conclusion:** The loss of chlorine residual in the distribution system allows for viral pathogens to survive transport from source to tap. Chlorine dosage is increased at the treatment facility.

#### **Case Study #4**

**A public beach is repeatedly being closed due to high enterococci counts. The significant loss of revenue prompts city officials to investigate the source(s) of the indicator bacteria.**

**Preliminary investigation:** An investigation of the beach and potential impacts reveals a sewage outfall close to the beach. Minimal stormwater runoff is observed. Domestic animals are not allowed on the beach and are not thought to be major contributors. Several older residences near the water are served by older sewer infrastructure. Some residences are on septic. Seagulls and other shore birds are frequently seen on the beach.

**Source tracking:** To eliminate or implicate human sewage as a source, water samples are screened for the human specific *Bacteroidales* and *Enterococcus* markers. The results are negative. The agency performing the source tracking suggests an *E. coli* and enterococci DNA fingerprinting study (using ribotyping; (125)) as the non-point potential sources are not readily identifiable. Several water samples are collected during low and high tide over a period of several days. The wind speed and degree of surf (calm, choppy) are noted during each sampling event. Core sand samples from the swash zone are collected. In addition, samples are collected from the wastewater treatment facility and the sewage outfall. Fecal samples are collected from a variety of shore birds and domestic animals (dogs). The sample collectors also notice an abundance of seaweed on the beach and collect several samples.

**Bacteriological test results:** Bacteriological testing confirms the high levels of *E. coli* and enterococci in the water column. Further analysis also shows levels to be 2-3 orders of magnitude higher in the water column during high tide with rough surf. The seaweed contains few microorganisms, but the sand contains several thousand microorganisms per 100 g.

**DNA fingerprinting:** Initial DNA fingerprinting analyses assign the fingerprint data into a broad classification of either “human derived” or “animal derived”. The results of this analysis indicate almost exclusive input from animal sources. This finding corroborates the molecular marker results obtained at the beginning of the study. DNA fingerprinting also reveals that a large proportion of the bacteria isolated from the sand samples share the same genetic profile. In addition, this profile is also the dominant fingerprint observed in the bacteria isolated from the water column. A cross-analysis of the data indicates that on the days with the most similarity between water and sand isolates, the surf was choppy and it was high tide. When unique fingerprints from water and sand isolates were grouped together, the majority of these fingerprints matched those obtained from seagull fecal samples. A very small proportion matched the sewage, outfall, and dog samples. Interestingly, the predominant “clonal” fingerprint did not match any of those generated from the fecal reference samples. Several fingerprints could not be classified and were labeled as “indeterminate”.

**Conclusion:** The high clonality of fingerprints observed in the sand and the water column indicates that the bacterial indicators may be re-growing in the sand. During high tide and/or rough surf, the sediment is disrupted and the bacteria are released into the water column. The source of the sediment isolates cannot be determined from this study because the clonal fingerprint did not match any fingerprints contained in the reference database; however, based on the other source tracking data (no human specific markers detected, fingerprints matching bird reference samples) a likely possibility is that many of these isolates are from animals. This

observation is further substantiated by the fact that the majority of isolates classified in this study were of avian origin. Human fecal pollution is not a likely source.

**Follow up:** During subsequent routine water quality monitoring, water samples are collected further offshore to minimize the impact of sediment bacteria and DNA fingerprinting is incorporated into the routine monitoring. Beach advisories and closings drop significantly the following year. Routine DNA fingerprinting consistently implicates birds as the source of the fecal indicators.

### **Microbial Source Tracking: What's Best?**

There is no single ideal source tracking method in use to date. Methods that are based on isolating indicator organisms are slow and labor-intensive, most have unacceptable levels of accuracy and false positives, and genetic evidence has shown that the population makeup of indicator organisms in the environment does not match fecal sources. Chemical methods and methods that are based on PCR of genetic markers do not have markers for all species and are not necessarily correlated with FIB. *No* method has adequately addressed the question of survival and storage of host-specific markers in the environment and their correlation with pathogens; even marker resistance to different standard waste treatments is poorly known. *No* method has adequately addressed the question of *proportional* survival of the species-specific markers or indicators; no method, whether culture-based, chemical, or based on QPCR, has been shown to work quantitatively over time. *Few* methods have been tested against blind samples and shown to accurately identify fecal inputs. Of the few that were tested in this way, only ribotyping, PFGE, and host-specific PCR were notably accurate.

The best evidence supports taking a multi-tiered approach (11, 90, 91, 117), moving from general to specific and from less to more expensive. After each step, progress can be assessed before deciding to move to the next one. The first step is always intensive sanitary surveys, to target sources spatially and temporally. Once "hot spots" are identified, their sources may be obvious even without any specific microbial source tracking. If not, then very directed source tracking can be done, starting with less-expensive methods, directed at identifying suspected sources or sources of concern. More expensive or more comprehensive methods would only be brought in to identify all species if needed. Appropriate rapid methods to distinguish human contamination would be chemical methods (caffeine, laundry brighteners and the like), host-specific PCR (*Bacteroidales* molecular markers, *E. coli* toxin genes, *Enterococcus* esp gene), and phage methods. The first tier of species identification could also be host-specific PCR, to identify common agricultural and domestic animals. The final tier, if needed, would be a library-based method to identify specific sources in more detail.

Overall, microbial source tracking methods should be viewed as tools that augment but do not replace current methods used for assessing water quality. All source tracking results should be interpreted carefully and in conjunction with other critical parameters such as local knowledge of potential fecal inputs and land use data. Finally, although a considerable amount of research supports the use of certain methods under certain circumstances, all water quality and source tracking studies should include the collection and analysis of appropriate field and quality control samples to ensure that a particular method or set of methods will provide the information necessary to answer the questions defined in any watershed assessment.

## References

1. **Albert, J. M., J. Munakata-Marr, L. Tenorio, and R. L. Siegrist.** 2003. Statistical evaluation of bacterial source tracking data obtained by rep-PCR DNA fingerprinting of *Escherichia coli*. *Environ Sci Technol* **37**:4554-60.
2. **Alderisio, K. A., and N. DeLuca.** 1999. Seasonal enumeration of fecal coliform bacteria from the feces of Ring-billed gulls (*Larus delawarensis*) and Canada geese (*Branta canadensis*). *Appl. Environ. Microbiol.* **65**:5628-5630.
3. **Allsop, K., and J. D. Stickler.** 1985. An assessment of *Bacteroides fragilis* group organisms as indicators of human faecal pollution. *J. Appl. Bacteriol.* **58**:95-99.
4. **Appelbee, A. J., R. C. Thompson, and M. E. Olson.** 2005. *Giardia* and *Cryptosporidium* in mammalian wildlife--current status and future needs. *Trends Parasitol* **21**:370-6.
5. **Bartlett, P. D.** 1987. Degradation of coprostanol in an experimental system. *Marine Pollution Bulletin* **18**:27-29.
6. **Bernhard, A. E., and K. G. Field.** 2000. A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. *Appl. Environ. Microbiol.* **66**:4571-4574.
7. **Bernhard, A. E., and K. G. Field.** 2000. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Appl. Environ. Microbiol.* **66**:1587-1594.
8. **Bernhard, A. E., T. Goyard, M. Simonich, and K. G. Field.** 2003. Application of a rapid method for identifying fecal pollution sources in a multi-use estuary. *Water Research* **37**:909-913.
9. **Betancourt, W. Q., and R. S. Fujioka.** 2006. *Bacteroides* spp. as reliable marker of sewage contamination in Hawaii's environmental waters using molecular techniques. *Water Sci Technol* **54**:101-7.
10. **Blanch, A. R., L. Belanche-Munoz, X. Bonjoch, J. Ebdon, C. Gantzer, F. Lucena, J. Ottoson, C. Kourtis, A. Iversen, I. Kuhn, L. Moce, M. Muniesa, J. Schwartzbrod, S. Skraber, G. T. Papageorgiou, H. Taylor, J. Wallis, and J. Jofre.** 2006. Integrated analysis of established and novel microbial and chemical methods for microbial source tracking. *Appl Environ Microbiol* **72**:5915-26.
11. **Boehm, A. B., J. A. Fuhrman, R. D. Morse, and S. B. Grant.** 2003. Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California. *Environ. Sci. Technol.* **37**:673-680.
12. **Bolin, C., C. Brown, and J. B. Rose.** 2004. Emerging zoonotic diseases and water, p. 19-25. *In* J. A. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D. O. Cliver, G. F. Craun, R. Fayer, and V. P. G. Gannon (ed.), *Waterborne Zoonoses*. World Health Organization, IWA Publishing, London.
13. **Bonadonna, L., R. Briancesco, M. Ottaviani, and E. Veschetti.** 2002. Occurrence of *Cryptosporidium* oocysts in sewage effluents and correlation with microbial, chemical, and physical water variables. *Env. Mon. Ass.* **75**:241-252.
14. **Bonjoch, X., E. Balleste, and A. R. Blanch.** 2005. Enumeration of bifidobacterial populations with selective media to determine the source of waterborne fecal pollution. *Water Res* **39**:1621-7.
15. **Bower, P. A., C. O. Scopel, E. T. Jensen, M. M. Depas, and S. L. McLellan.** 2005. Detection of genetic markers of fecal indicator bacteria in Lake Michigan and determination of their relationship to *Escherichia coli* densities using standard microbiological methods. *Appl Environ Microbiol* **71**:8305-13.

16. **Buerge, II, T. Poiger, M. D. Muller, and H. R. Buser.** 2003. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. *Environ Sci Technol* **37**:691-700.
17. **Bull, I. D., M. J. Lockheart, M. M. Elhmmali, D. J. Roberts, and R. P. Evershed.** 2002. The origin of faeces by means of biomarker detection. *Environ Int* **27**:647-54.
18. **Burkhardt, M. R., P. P. Soliven, S. L. Werner, and D. G. Vaught.** 1999. Determination of submicrogram-per-liter concentrations of caffeine in surface water and groundwater samples by solid-phase extraction and liquid chromatography. *J. AOAC International* **82**:161-166.
19. **Byappanahalli, M., M. Fowler, D. Shively, and R. Whitman.** 2003. Ubiquity and persistence of *Escherichia coli* in a Midwestern coastal stream. *Appl Environ Microbiol* **69**:4549-55.
20. **Byappanahalli, M., and R. Fujioka.** 2004. Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. *Water Sci Technol* **50**:27-32.
21. **Byappanahalli, M. N., R. L. Whitman, D. A. Shively, M. J. Sadowsky, and S. Ishii.** 2006. Population structure, persistence, and seasonality of autochthonous *Escherichia coli* in temperate, coastal forest soil from a Great Lakes watershed. *Environ Microbiol* **8**:504-13.
22. **Byappanahalli, M. N., R. L. Whitman, D. A. Shively, W. T. Ting, C. C. Tseng, and M. B. Nevers.** 2006. Seasonal persistence and population characteristics of *Escherichia coli* and enterococci in deep backshore sand of two freshwater beaches. *J Water Health* **4**:313-20.
23. **Carillo, M., E. Estrada, and T. C. Hazen.** 1985. Survival and enumeration of the fecal indicators *Bifidobacterium adolescentis* and *Escherichia coli* in a tropical rain forest watershed. *Appl. Environ. Microbiol.* **50**:468-476.
24. **Carroll, S., M. Hargreaves, and A. Goonetilleke.** 2005. Sourcing fecal pollution from onsite wastewater treatment systems in surface waters using antibiotic resistance analysis. *J Appl Microbiol* **99**:471-.
25. **Carson, C. A., J. M. Christiansen, H. Yampara-Iquise, V. W. Benson, C. Baffaut, J. V. Davis, R. R. Broz, W. B. Kurtz, W. M. Rogers, and W. H. Fales.** 2005. Specificity of a *Bacteroides thetaiotaomicron* marker for human feces. *Appl Environ Microbiol* **71**:4945-9.
26. **Carson, C. A., B. L. Shear, M. R. Ellershiek, and A. Asfaw.** 2001. Identification of fecal *Escherichia coli* from humans and animals by ribotyping. *Appl. Environ. Microbiol.* **67**:1503-1507.
27. **Carson, C. A., B. L. Shear, M. R. Ellershiek, and J. D. Schnell.** 2003. Comparison of ribotyping and repetitive extragenic palindromic-PCR for identification of fecal *Escherichia coli* from humans and animals. *Appl Environ Microbiol* **69**:1836-1839.
28. **Caugant, D. A., B. R. Levin, and R. K. Selander.** 1984. Distribution of multilocus genotypes of *Escherichia coli* within and between host families. *J Hyg (Lond)* **92**:377-84.
29. **Chapron, C. D., N. A. Ballester, J. H. Fontaine, C. N. Frades, and A. B. Margolin.** 2000. Detection of astroviruses, enteroviruses, and adenovirus types 40 and 41 in surface waters collected and evaluated by the information collection rule and an integrated cell culture-nested PCR procedure. *Appl Environ Microbiol* **66**:2520-5.
30. **Chern, E. C., Y. L. Tsai, and B. H. Olson.** 2004. Occurrence of genes associated with enterotoxigenic and enterohemorrhagic *Escherichia coli* in agricultural waste lagoons. *Appl Environ Microbiol* **70**:356-62.
31. **Cheung, W. H. S. (ed.).** 1988. Health effects of beach water pollution in Hong Kong, vol. 88. Vincent Blue Copy Co., Hong Kong.

32. **Coburn, P. S., A. S. Baghdayan, G. Dolan, and N. Shankar.** 2006. Horizontal transfer of virulence genes encoded on the *Enterococcus faecalis* pathogenicity island. *Mol Microbiol.*
33. **Cole, D., S. C. Long, and M. D. Sobsey.** 2003. Evaluation of F+ RNA and DNA coliphages as source-specific indicators of fecal contamination in surface waters. *Appl. Environ. Microbiol.* **69**:6507-6514.
34. **Colford, J. M., Jr., T. J. Wade, K. C. Schiff, C. C. Wright, J. F. Griffith, S. K. Sandhu, S. Burns, M. Sobsey, G. Lovelace, and S. B. Weisberg.** 2007. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology* **18**:27-35.
35. **Dick, L. K., A. E. Bernhard, T. J. Brodeur, J. W. Santo Domingo, J. M. Simpson, S. P. Walters, and K. G. Field.** 2005. Host distributions of uncultivated fecal *Bacteroidales* reveal genetic markers for fecal source identification. *Appl Environ Microbiol* **71**:3184-3191.
36. **Dick, L. K., and K. G. Field.** 2004. Rapid estimation of numbers of fecal *Bacteroidetes* by use of a quantitative PCR assay for 16S rRNA genes. *Appl Environ Microbiol* **70**:5695-5697.
37. **Dick, L. K., M. T. Simonich, and K. G. Field.** 2005. Microplate subtractive hybridization to enrich for source-specific *Bacteroidales* fecal pollution indicators. *Appl Environ Microbiol* **71**:3179-3183.
38. **Dombek, P. E., L. K. Johnson, S. T. Zimmerley, and M. J. Sadowsky.** 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. *Appl. Environ. Microbiol.* **66**:2572-2577.
39. **Duran, M., B. Z. Haznedaroglu, and D. H. Zitomer.** 2006. Microbial source tracking using host specific FAME profiles of fecal coliforms. *Water Res* **40**:67-74.
40. **Dureth, S., H. Reimer, and K. Pecher.** 1986. Tracing faecal pollution by coprostanol and intestinal bacteria in an ice covered Finnish lake loaded with both industrial and domestic sewage. *Water Air Soil Poll.* **28**:131-149.
41. **Eckburg, P. B., E. M. Bik, C. N. Bernstein, E. Purdom, L. Dethlefsen, M. Sargent, S. R. Gill, K. E. Nelson, and D. A. Relman.** 2005. Diversity of the human intestinal flora. *Science.*
42. **Edge, T. A., and S. Hill.** 2005. Occurrence of antibiotic resistance in *Escherichia coli* from surface waters and fecal pollution sources near Hamilton, Ontario. *Can J Microbiol* **51**:501-5.
43. **Edwards, D. D., G. A. McFeters, and M. I. Venkatesan.** 1998. Distribution of *Clostridium perfringens* and fecal sterols in a benthic coastal marine environment influenced by the sewage outfall from McMurdo Station. *Appl. Environ. Microbiol.* **41**:1139-1143.
44. **Eganhouse, R. P., D. L. Blumfield, and I. R. Kaplan.** 1983. Long-chain alkylbenzenes as molecular tracers of domestic wastes in the marine environment. *Environ. Sci. Technol.* **17**:523-530.
45. **Eganhouse, R. P., and P. M. Sherblom.** 2001. Anthropogenic organic contaminants in the effluent of a combined sewer overflow: impact on Boston Harbor. *Mar Environ Res* **51**:51-74.
46. **Ferguson, R. L., E. N. Buckley, and A. V. Palumbo.** 1984. Response of marine bacterioplankton to differential filtration and confinement. *Appl. Environ. Microbiol.* **47**:49-55.
47. **Field, K. G.** 2004. Faecal Source Identification. *In* J. A. Cotruvo, A. Dufour, G. Rees, J. Bartram, R. Carr, D. O. Cliver, G. F. Craun, R. Fayer, and V. P. G. Gannon (ed.), *Waterborne Zoonoses: Identification, Causes and Control.* World Health Organization, IWA Publishing Alliance House, London.

48. **Field, K. G., E. C. Chern, L. K. Dick, J. A. Fuhrman, J. F. Griffith, P. A. Holden, M. G. LaMontagne, J. Le, B. H. Olson, and M. T. Simonich.** 2003. A comparative study of culture-independent, library-independent genotypic methods of fecal source tracking. *J. Water Health* **1**:181-194.
49. **Fiksdal, L., J. S. Make, S. J. LaCroix, and J. T. Staley.** 1985. Survival and detection of *Bacteroides* spp., prospective indicator bacteria. *Appl. Environ. Microbiol.* **49**:148-150.
50. **Fogarty, L. R., S. K. Haack, M. J. Wolcott, and R. L. Whitman.** 2003. Abundance and characteristics of the recreational water quality indicator bacteria *Escherichia coli* and enterococci in gull faeces. *J. Appl. Microbiol.* **94**:865-878.
51. **Fong, T. T., D. W. Griffin, and E. K. Lipp.** 2005. Molecular assays for targeting human and bovine enteric viruses in coastal waters and their application for library-independent source tracking. *Appl Environ Microbiol* **71**:2070-8.
52. **Fong, T. T., and E. K. Lipp.** 2005. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiol Mol Biol Rev* **69**:357-71.
53. **Fujioka, R., C. Dian-Denton, J. Castro, and K. Morphew.** 1999. Soil: the environmental source of *Escherichia coli* and Enterococci in Guam's streams. *J. Appl. Microbiol.* **85**:83S-89S.
54. **Furuse, K. A., A. Andro, S. Osawa, and I. Watanabe.** 1981. Distribution of ribonucleic acid coliphages in raw sewage from treatments in Japan. *Appl. Environ. Microbiol.* **41**:1139-1143.
55. **Gantzer, C., A. Maul, J. M. Audic, and L. Schwartzbrod.** 1998. Detection of infectious enteroviruses, enterovirus genomes, somatic coliphages, and *Bacteroides fragilis* phages in treated wastewater. *Appl Environ Microbiol* **64**:4307-12.
56. **Geldenhuis, J. C., and P. D. Pretorius.** 1989. The occurrence of enteric viruses in polluted water, correlation to indicator organisms, and factors influencing their numbers. *Water Sci Technol* **21**:105-9.
57. **Geldreich, E. E.** 1978. Bacterial populations and indicator concepts in feces, sewage, stormwater, and solid wastes. *In* G. Berg (ed.), *Indicators of viruses in water and food.* Ann Arbor Science Publishers, Ann Arbor.
58. **Geldreich, E. E.** 1976. Fecal coliform and fecal streptococcus density relationships in water discharges and receiving waters. *CRC Critical Rev Environ Cont*:349-369.
59. **Gilpin, B., T. James, F. Nourozi, D. Saunders, P. Scholes, and M. Savill.** 2003. The use of chemical and molecular microbial indicators for faecal source identification. *Water Science and Technology* **47**:39-43.
60. **Gordon, D. M., and A. Cowling.** 2003. The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. *Microbiology* **149**:3575-86.
61. **Griffin, D. W., K. A. Donaldson, J. H. Paul, 3rd, and J. B. Rose.** 2003. Pathogenic human viruses in coastal waters. *Clinical Microbiol. Rev.* **16**:129-143.
62. **Griffin, D. W., C. J. Gibson, 3rd, E. K. Lipp, K. Riley, J. H. Paul, 3rd, and J. B. Rose.** 1999. Detection of viral pathogens by reverse transcriptase PCR and of microbial indicators by standard methods in the canals of the Florida Keys. *Appl Environ Microbiol* **65**:4118-25.
63. **Griffith, J. F., S. B. Weisburg, and C. D. McGee.** 2003. Evaluation of microbial source tracking methods using mixed fecal sources in aqueous test samples. *J. Water Health* **1**:141-152.
64. **Guan, S., R. Xu, S. Chen, J. Odumeru, and C. L. Gyles.** 2002. Development of a procedure for discriminating among *Escherichia coli* isolates from animal and human sources. *Appl. Environ. Microbiol.* **68**:2690-2698.

65. **Hagedorn, C., S. L. Robinson, J. R. Filtz, S. M. Grubbs, T. A. Angier, and R. B. Reneau Jr.** 1999. Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci. *Appl Environ Microbiol* **65**:5522-31.
66. **Hartel, P. G., J. D. Summer, J. L. Hill, J. V. Collins, J. A. Entry, and W. I. Segars.** 2002. Geographic variability of *Escherichia coli* ribotypes from animals in Idaho and Georgia. *J. Environ. Qual.* **31**:1273-1278.
67. **Hartly, C. L., K. Howne, A. H. Linton, K. B. Linton, and M. H. Richmond.** 1975. Distribution of R plasmids among O-antigen types of *Escherichia coli* isolated from human and animal sources. *Antimicrob Agents Chemother* **8**:122-131.
68. **Harwood, V. J., J. Butler, D. Parrish, and V. Wagner.** 1999. Isolation of fecal coliform bacteria from the diamondback terrapin (*Malaclemys terrapin centrata*). *Appl. Environ. Microbiol.* **65**:865-867.
69. **Harwood, V. J., A. D. Levine, T. M. Scott, V. Chivukula, J. Lukasik, S. R. Farrah, and J. B. Rose.** 2005. Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and Environmental Microbiology* **71**:3163-3170.
70. **Harwood, V. J., J. Whitlock, and V. Withington.** 2000. Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical waters. *Appl Environ Microbiol* **66**:3698-704.
71. **Harwood, V. J., B. A. Wiggins, C. Hagedorn, R. D. Ellender, J. Gooch, J. Kern, M. Samadpour, A. C. H. Chapman, and B. J. Robinson.** 2003. Phenotypic library-based microbial source tracking methods: efficacy in the California collaborative study. *J. Water Health* **1**:153-166.
72. **Hassan, W. M., S. Y. Wang, and R. D. Ellender.** 2005. Methods to increase fidelity of repetitive extragenic palindromic PCR fingerprint-based bacterial source tracking efforts. *Appl Environ Microbiol* **71**:512-8.
73. **Haznedaroglu, B. Z., D. H. Zitomer, G. B. Hughes-Strange, and M. Duran.** 2005. Whole-cell fatty-acid composition of total coliforms to predict sources of fecal contamination. *J. Environ. Eng.* **131**:1426-1432.
74. **Holmes, P. R.** 1989. Research into health risks at bathing beaches in Hong Kong. *J. Inst. Water Environ. Manage.* **3**:488-495.
75. **Horman, A., R. Rimhanen-Finne, L. Maunula, C.-H. von Bonsdorff, N. Torvela, A. Heikinheimo, and M.-L. Hanninen.** 2004. *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., Noroviruses, and indicator organisms in surface water in southwestern Finland, 2000-2001. *Appl. Environ. Microbiol.* **70**:87-95.
76. **Hundesda, A., C. Maluquer de Motes, S. Bofill-Mas, N. Albinana-Gimenez, and R. Girones.** 2006. Identification of human and animal adenoviruses and polyomaviruses for determination of sources of fecal contamination in the environment. *Appl Environ Microbiol* **72**:7886-93.
77. **Indest, K. J., K. Betts, and J. S. Furey.** 2005. Application of oligonucleotide microarrays for bacterial source tracking of environmental *Enterococcus* sp. isolates. *Int J Environ Res Public Health* **2**:175-85.
78. **Ishii, S., W. B. Ksoll, R. E. Hicks, and M. J. Sadowsky.** 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Appl Environ Microbiol* **72**:612-21.
79. **Isobe, K. O., M. Tarao, M. P. Zakaria, N. H. Chiem, L. Y. Minh, and H. Takada.** 2002. Quantitative application of fecal sterols using gas-chromatography-mass spectrometry to investigate fecal pollution in tropical waters: western Malaysia and Mekong Delta, Vietnam. *Environ. Sci. Technol.* **36**:4497-4507.

80. **Jenkins, T. M., T. M. Scott, M. R. Morgan, and J. B. Rose.** 2005. Occurrence of alternative fecal indicators and enteric viruses in Michigan rivers. *J. Great Lakes Res.* **31**:22-31.
81. **Jiang, S., R. Noble, and W. Chu.** 2001. Human adenoviruses and coliphages in urban runoff-impacted coastal waters of Southern California. *Appl Environ Microbiol* **67**:179-84.
82. **Jimenez-Clavero, M. A., E. Escribano-Romero, C. Mansilla, N. Gomez, L. Cordoba, N. Roblas, F. Ponz, V. Ley, and J. C. Saiz.** 2005. Survey of bovine enterovirus in biological and environmental samples by a highly sensitive real-time reverse transcription-PCR. *Appl Environ Microbiol* **71**:3536-43.
83. **Jimenez-Clavero, M. A., C. Fernandez, J. A. Ortiz, J. Pro, G. Carbonell, J. V. Tarazona, N. Roblas, and V. Ley.** 2003. Teschoviruses as indicators of porcine fecal contamination of surface water. *Appl Environ Microbiol* **69**:6311-5.
84. **Johnson, L. K., M. B. Brown, E. A. Carruthers, J. A. Ferguson, P. E. Dombek, and M. J. Sadowsky.** 2004. Sample size, library composition, and genotypic diversity among natural populations of *Escherichia coli* from different animals influence accuracy of determining sources of fecal pollution. *Appl Environ Microbiol* **70**:4478-85.
85. **Khatib, L. A., Y. L. Tsai, and B. H. Olson.** 2002. A biomarker for the identification of cattle fecal pollution in water using the LTIIa toxin gene from enterotoxigenic *E. coli*. *Appl. Microbiol. Biotechnol.* **59**:97-104.
86. **Khatib, L. A., Y. L. Tsai, and B. H. Olson.** 2003. A biomarker for the identification of swine fecal pollution in water using the STII toxin gene from enterotoxigenic *E. coli*. *Appl. Microbiol. Biotechnol.* **63**:231-238.
87. **Kinzelman, J., S. L. McLellan, A. D. Daniels, S. Cashin, A. Singh, S. Gradus, and R. Bagley.** 2004. Non-point source pollution: determination of replication versus persistence of *Escherichia coli* in surface water and sediments with correlation of levels to readily measurable environmental parameters. *J Water Health* **2**:103-14.
88. **Kreader, C. A.** 1995. Design and evaluation of *Bacteroides* DNA probes for the specific detection of human fecal pollution. *Appl. Environ. Microbiol.* **61**:1171-1179.
89. **Kreader, C. A.** 1998. Persistence of PCR-detectable *Bacteroides distasonis* from human feces in river water. *Appl. Environ. Microbiol.* **64**:4103-4105.
90. **Kuntz, R. L., P. G. Hartel, D. G. Godfrey, J. L. McDonald, K. W. Gates, and W. I. Segars.** 2003. Targeted sampling protocol as prelude to bacterial source tracking with *Enterococcus faecalis*. *J Environ Qual* **32**:2311-8.
91. **Kuntz, R. L., P. G. Hartel, K. Rodgers, and W. I. Segars.** 2004. Presence of *Enterococcus faecalis* in broiler litter and wild bird feces for bacterial source tracking. *Water Res* **38**:3551-7.
92. **Lamendella, R., J. W. Domingo, D. B. Oerther, J. R. Vogel, and D. M. Stoeckel.** 2006. Assessment of fecal pollution sources in a small northern-plains watershed using PCR and phylogenetic analyses of *Bacteroidetes* 16S rRNA gene. *FEMS Microbiol Ecol.*
93. **Layton, A., L. McKay, D. Williams, V. Garrett, R. Gentry, and G. Saylor.** 2006. Development of *Bacteroides* 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Appl Environ Microbiol* **72**:4214-24.
94. **Leavis, H., J. Top, N. Shankar, K. Borgen, M. Bonten, J. van Embden, and R. J. Willems.** 2004. A novel putative enterococcal pathogenicity island linked to the *esp* virulence gene of *Enterococcus faecium* and associated with epidemicity. *J Bacteriol* **186**:672-82.
95. **Leeming, R., A. Bakk, N. Ashbolt, and P. Nichols.** 1996. Using fecal sterols from humans and animals to distinguish fecal pollution in receiving waters. *Water Res* **30**:2893-2900.

96. **Lemarchand, K., and P. Lebaron.** 2003. Occurrence of *Salmonella* spp. and *Cryptosporidium* spp. in a French coastal watershed: relationship with fecal indicators. *FEMS Microbiol Letts* **218**:203-209.
97. **Leung, K. T., R. Mackereth, Y.-C. Tien, and E. Topp.** 2004. A comparison of AFLP and ERIC-PCR analyses for discriminating *Escherichia coli* from cattle, pig and human sources. *FEMS Microbiol. Ecol.* **47**:111-119.
98. **Ley, V., J. Higgins, and R. Fayer.** 2002. Bovine enteroviruses as indicators of fecal contamination. *Appl Environ Microbiol* **68**:3455-3461.
99. **Liu, L., M. S. Phanikumar, S. L. Molloy, R. L. Whitman, D. A. Shively, M. B. Nevers, D. J. Schwab, and J. B. Rose.** 2006. Modeling the transport and inactivation of *E. coli* and enterococci in the near-shore region of Lake Michigan. *Environ Sci Technol* **40**:5022-8.
100. **Long, S. C., and M. D. Sobsey.** 2004. A comparison of the survival of F+RNA and F+DNA coliphages in lake water microcosms. *J Water Health* **2**:15-22.
101. **Lund, V.** 1996. Evaluation of *E. coli* as an indicator for the presence of *Campylobacter jejuni* and *Yersinia enterocolitica* in chlorinated and untreated oligotrophic lake water. *Wat. Res.* **30**:1528-1534.
102. **Lynch, P. A., B. J. Gilpin, L. W. Sinton, and M. G. Savill.** 2002. The detection of *Bifidobacterium adolescentis* by colony hybridization as an indicator of human faecal pollution. *J Appl Microbiol* **92**:526-33.
103. **Maluquer de Motes, C., P. Clemente-Casares, A. Hundesa, M. Martin, and R. Girones.** 2004. Detection of bovine and porcine adenoviruses for tracing the source of fecal contamination. *Appl Environ Microbiol* **70**:1448-54.
104. **Mara, D. D., and J. Oragui.** 1985. Bacteriological methods for distinguishing between human and animal faecal pollution of water: results of fieldwork in Nigeria and Zimbabwe. *Bull World Health Organ* **63**:773-83.
105. **Mara, D. D., and J. I. Oragui.** 1983. Sorbitol-fermenting bifidobacteria as specific indicators of human fecal pollution. *J. Appl. Bacteriol.* **55**:349-357.
106. **McBride, G. B., C. E. Salmond, D. R. Bandaranayake, S. J. Turner, G. D. Lewis, and D. G. Till.** 1998. Health effects of marine bathing in New Zealand. *Int. J. Environ. Hlth. Res* **8**:173-189.
107. **McLellan, S. L.** 2004. Genetic diversity of *Escherichia coli* isolated from urban rivers and beach water. *Appl Environ Microbiol* **70**:4658-4665.
108. **McLellan, S. L., A. D. Daniels, and A. K. Salmore.** 2003. Genetic characterization of *Escherichia coli* populations from host sources of fecal pollution by using DNA fingerprinting. *Appl. Environ. Microbiol.* **69**:2587-2594.
109. **McQuaig, S. M., T. M. Scott, V. J. Harwood, S. R. Farrah, and J. O. Lukasik.** 2006. Detection of human-derived fecal pollution in environmental waters by use of a PCR-based human polyomavirus assay. *Appl Environ Microbiol* **72**:7567-74.
110. **Meays, C. L., K. Broersma, R. Nordin, and A. Mazumder.** 2004. Source tracking fecal bacteria in water: a critical review of current methods. *J Environ Manage* **73**:71-9.
111. **Moore, D. F., V. J. Harwood, D. M. Ferguson, J. Lukasik, P. Hannah, M. Getrich, and M. Brownell.** 2005. Evaluation of antibiotic resistance analysis and ribotyping for identification of fecal pollution sources in an urban watershed. *J Appl Microbiol* **99**:618-.
112. **Myoda, S. P., C. A. Carson, J. J. Fuhrmann, B.-K. Hahm, P. G. Hartel, R. L. Kuntz, C. H. Nakatsu, M. J. Sadowsky, M. Samadpour, and H. Yampara-Iquise.** 2003. Comparing genotypic bacterial source tracking methods that require a host origin database. *J. Water Health* **1**:167-180.
113. **Nebra, Y., X. Bonjoch, and A. R. Blanch.** 2003. Use of *Bifidobacterium dentium* as an indicator of the origin of fecal water pollution. *Appl Environ Microbiol* **69**:2651-2656.

114. **Nichols, P. D., R. Leeming, M. S. Rayner, V. Latham, N. J. Ashbolt, and C. Turner.** 1993. Comparison of the abundance of the fecal sterol coprostanol and fecal bacterial groups in inner-shelf waters and sediments near Sydney, Australia. *J. Chromatography* **64**:189-195.
115. **Noble, R. T., S. M. Allen, A. D. Blackwood, W. Chu, S. C. Jiang, G. L. Lovelace, M. D. Sobsey, J. R. Stewart, and D. A. Wait.** 2003. Use of viral pathogens and indicators to differentiate between human and non-human fecal contamination in a microbial source tracking comparison study. *J. Water Health* **1**:195-208.
116. **Noble, R. T., and J. A. Fuhrman.** 2001. Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: low correlation to bacterial indicator levels. *Hydrobiologia* **460**:175-184.
117. **Noble, R. T., J. F. Griffith, A. D. Blackwood, J. A. Fuhrman, J. B. Gregory, X. Hernandez, X. Liang, A. A. Bera, and K. Schiff.** 2006. Multitiered approach using quantitative PCR to track sources of fecal pollution affecting Santa Monica Bay, California. *Appl Environ Microbiol* **72**:1604-12.
118. **Oancea, C., I. Klare, W. Witte, and G. Werner.** 2004. Conjugative transfer of the virulence gene, *esp*, among isolates of *Enterococcus faecium* and *Enterococcus faecalis*. *J Antimicrob Chemother* **54**:232-5.
119. **Okabe, S., N. Okayama, O. Savichtcheva, and T. Ito.** 2006. Quantification of host-specific *Bacteroides-Prevotella* 16S rRNA genetic markers for assessment of fecal pollution in freshwater. *Appl Microbiol Biotechnol*.
120. **Olapade, O. A., M. M. Depas, E. T. Jensen, and S. L. McLellan.** 2006. Microbial communities and fecal indicator bacteria associated with *Cladophora* mats on beach sites along Lake Michigan shores. *Appl Environ Microbiol* **72**:1932-8.
121. **Oragui, J. I., and D. D. Mara.** 1983. Investigation of the survival characteristics of *Rhodococcus coprophilis* and certain fecal indicator bacteria. *Appl Environ Microbiol* **46**.
122. **Oshiro, R. K., and B. H. Olson.** 1997. Occurrence of STh toxin gene in wastewater. In D. Kay and C. Fricher (ed.), *Coliforms and E. coli: Problem or Solution?* The Royal Society of Chemistry, Cambridge, England.
123. **Parveen, S., N. C. Hodge, R. E. Stall, S. R. Farrah, and M. L. Tamplin.** 2001. Genotypic and phenotypic characterization of human and non-human *Escherichia coli*. *Water Res* **35**:379-386.
124. **Parveen, S., R. L. Murphree, L. Edmiston, C. W. Kaspar, K. M. Portier, and M. L. Tamplin.** 1997. Association of multiple-antibiotic-resistance profiles with point and nonpoint sources of *Escherichia coli* in Apalachicola Bay. *Appl Environ Microbiol* **63**:2607-2612.
125. **Parveen, S., K. M. Portier, K. Robinson, L. Edmiston, and M. Tamplin.** 1999. Discriminate analysis of ribotype profiles of *Escherichia coli* for differentiating human and nonhuman sources of fecal pollution. *Appl. Environ. Microbiol.* **65**:3142-3147.
126. **Payan, A., J. Ebdon, H. Taylor, C. Gantzer, J. Ottoson, G. T. Papageorgiou, A. R. Blanch, F. Lucena, J. Jofre, and M. Muniesa.** 2005. Method for isolation of *Bacteroides* bacteriophage host strains suitable for tracking sources of fecal pollution in water. *Appl Environ Microbiol* **71**:5659-62.
127. **Pina, S., M. Puig, F. Lucena, J. Jofre, and R. Girones.** 1998. Viral pollution in the environment and in shellfish: human adenovirus detection by PCR as an index of human viruses. *Appl. Environ. Microbiol.* **64**:3376-3382.
128. **Power, M. L., J. Littlefield-Wyer, D. M. Gordon, D. A. Veal, and M. B. Slade.** 2005. Phenotypic and genotypic characterization of encapsulated *Escherichia coli* isolated from blooms in two Australian lakes. *Environ Microbiol* **7**:631-640.

129. **Puglisi, E., M. Nicelli, E. capri, M. Trevisan, and A. A. Del Re.** 2003. Cholesterol, beta-sitosterol, ergosterol, and coprostanol in agricultural soils. *J. Environ. Qual.* **32**:466-471.
130. **Puig, A., N. Queralt, J. Jofre, and R. Araujo.** 1999. Diversity of *Bacteroides fragilis* strains in their capacity to recover phages from human and animal wastes and from fecally polluted wastewater. *Appl Environ Microbiol* **65**:1772-6.
131. **Pusch, D., D. Y. Oh, S. Wolf, R. Dumke, U. Schroter-Bobsin, M. Hohne, I. Roske, and E. Schreier.** 2005. Detection of enteric viruses and bacterial indicators in German environmental waters. *Arch Virol* **150**:929-47.
132. **Resnick, I. G., and M. A. Levin.** 1981. Assessment of Bifidobacteria as indicators of human fecal pollution. *Appl. Environ. Microbiol.* **42**:433-438.
133. **Rhodes, M. W., and H. Kator.** 1999. Sorbitol-fermenting bifidobacteria as indicators of diffuse human fecal pollution in estuarine watersheds. *J. Appl. Microbiol.* **87**:528-535.
134. **Rowbotham, T. J., and T. Cross.** 1977. Ecology of *Rhodococcus coprophilus* and associated actinomycetes in fresh water and agricultural habitats. *J Gen Microbiol* **100**:231-240.
135. **Samadpour, M., and N. Chechowitz.** 1995. Little Soos Creek Microbial Source Tracking. Kings County Department of Public Works.
136. **Samadpour, M., M. C. Roberts, C. L. Kitts, W. Mulugeta, and D. Alfi.** 2005. The use of ribotyping and antibiotic resistance patterns for identification of host sources of *Escherichia coli* strains. *Lett Appl Microbiol* **40**:63-8.
137. **Savill, M. G., S. R. Murray, P. Scholes, E. W. Maas, R. E. McCormick, E. B. Moore, and B. J. Gilpin.** 2001. Application of polymerase chain reaction (PCR) and TaqMan PCR techniques to the detection and identification of *Rhodococcus coprophilus* in faecal samples. *J Microbiol Methods* **47**:355-68.
138. **Scott, L., P. McGee, J. J. Sheridan, B. Earley, and N. Leonard.** 2006. A comparison of the survival in feces and water of *Escherichia coli* O157:H7 grown under laboratory conditions or obtained from cattle feces. *J Food Prot* **69**:6-11.
139. **Scott, T. M., J. B. Rose, T. M. Jenkins, S. R. Farrah, and J. Lukasik.** 2002. Microbial source tracking: current methodology and future directions. *Appl. Environ. Microbiol.* **68**:5796-5803.
140. **Scott, T. M., T. M. Jenkins, J. Lukasik, and J. B. Rose.** 2005. Potential use of a host associated molecular marker in *Enterococcus faecium* as an index of human pollution. *Environ Sci Technol* **39**.
141. **Scott, T. M., S. Parveen, K. M. Portier, J. B. Rose, M. L. Tamplin, S. A. Farrah, A. Koo, and J. Lukasik.** 2003. Geographical variation in ribotype profiles of *Escherichia coli* isolates from humans, swine, poultry, beef, and dairy cattle in Florida. *Appl Environ Microbiol* **69**:1089-1092.
142. **Seurinck, S., T. Defoirdt, W. Verstraete, and S. D. Siciliano.** 2005. Detection and quantification of the human-specific HF183 *Bacteroides* 16S rRNA genetic markers with realtime PCR for assessment of human fecal pollution in freshwater. *Environ Microbiol* **7**:249-259.
143. **Seurinck, S., E. Deschepper, B. Deboch, W. Verstraete, and S. Siciliano.** 2006. Characterization of *Escherichia coli* isolates from different fecal sources by means of classification tree analysis of Fatty Acid methyl ester (fame) profiles. *Environ Monit Assess* **114**:433-45.
144. **Seurinck, S., W. Verstraete, and S. Siciliano.** 2005. Microbial source tracking for identification of fecal pollution. *Rev. Environ. Sci. BioTech.* **4**:19-37.
145. **Shanks, O. C., C. Nietch, M. T. Simonich, M. Younger, D. Reynolds, and K. G. Field.** 2006. A basin-wide analysis of the dynamics of fecal contamination and fecal source identification in Tillamook Bay, Oregon. *Appl Environ Microbiol* **72**:5537-5546.

146. **Shanks, O. C., J. W. Santo Domingo, R. Lamendella, C. A. Kelty, and J. E. Graham.** 2006. Competitive metagenomic DNA hybridization identifies host-specific microbial genetic markers in cow fecal samples. *Appl Environ Microbiol* **72**:4054-60.
147. **Sigler, V., and L. Pasutti.** 2006. Evaluation of denaturing gradient gel electrophoresis to differentiate *Escherichia coli* populations in secondary environments. *Environ Microbiol* **8**:1703-11.
148. **Simpson, J. M., J. W. Santo Domingo, and D. J. Reasoner.** 2002. Microbial source tracking: state of the science. *Environ. Sci. Technol.* **36**:5279 -5288.
149. **Sinton, L. W., A. M. Donnison, and C. M. Hastie.** 1993. Faecal streptococci as faecal pollution indicators: a review. Part II: Sanitary significance, survival, and use. *New Zealand J. Marine Freshwater Res.* **27**:117-137.
150. **Sinton, L. W., R. K. Finlay, and D. J. Hannah.** 1998. Distinguishing human from animal fecal contamination in water: a review. *N. Z. J. Mar. Freshwater Res.* **32**:323-348.
151. **Sosiak, A., and J. Dixon.** 2006. Impacts on water quality in the upper Elbow River. *Water Sci Technol* **53**:309-16.
152. **Souza, V., M. Rocha, A. Valera, and L. E. Eguiarte.** 1999. Genetic structure of natural populations of *Escherichia coli* in wild hosts on different continents. *Appl Environ Microbiol* **65**:3373-85.
153. **Standley, L. J., L. A. Kaplan, and D. Smith.** 2000. Molecular tracers of organic matter sources to surface water resources. *Environ. Sci. Technol.* **34**:3124-3130.
154. **Stewart-Pullaro, J., J. W. Daugomah, D. E. Chestnut, D. A. Graves, M. D. Sobsey, and G. I. Scott.** 2006. F+ RNA coliphage typing for microbial source tracking in surface waters. *J Appl Microbiol* **101**:1015-26.
155. **Stoeckel, D. M., M. V. Mathes, K. E. Hyer, C. Hagedorn, H. Kator, J. Lukasik, T. L. O'Brien, T. W. Fenger, M. Samadpour, K. M. Strickler, and B. A. Wiggins.** 2004. Comparison of seven protocols to identify fecal contamination sources using *Escherichia coli*. *Environ Sci Technol* **38**:6109-17.
156. **Straub, T. M., and D. P. Chandler.** 2003. Towards a unified system for detecting waterborne pathogens. *J. Microbiol. Methods* **53**:185-197.
157. **Suprihatin, I., H. Fallowfield, R. Bentham, and N. Cromar.** 2003. Determination of faecal pollutants in Torrens and Patawalonga catchment waters in South Australia using faecal sterols. *Water Sci. Technol.* **47**:283-289.
158. **Surbeck, C. Q., S. C. Jiang, J. H. Ahn, and S. B. Grant.** 2006. Flow fingerprinting fecal pollution and suspended solids in stormwater runoff from an urban coastal watershed. *Environ Sci Technol* **40**:4435-41.
159. **Takada, H., and R. P. Eganhouse.** 1998. Molecular markers of anthropogenic waste, p. 2883-2940. *In* R. A. Meyers (ed.), *Encyclopedia of environmental analysis and remediation*. John Wiley, New York.
160. **Tartera, C., and J. Jofre.** 1987. Bacteriophages active against *Bacteroides fragilis* in sewage polluted waters. *Appl. Environ. Microbiol.* **53**:1632-1637.
161. **Tartera, C., F. Lucena, and J. Jofre.** 1989. Human origin of *Bacteroides fragilis* bacteriophages present in the environment. *Appl. Environ. Microbiol.* **55**:2696-2701.
162. **Tsai, Y. L., J. Y. Le, and B. H. Olson.** 2003. Magnetic bead hybridization to detect enterotoxigenic *Escherichia coli* strains associated with cattle in environmental water sources. *Can J Microbiol* **49**:391-8.
163. **Tsai, Y. L., M. D. Sobsey, L. R. Sangermano, and C. J. Palmer.** 1993. Simple method of concentrating enteroviruses and hepatitis A virus from sewage and ocean water for rapid detection by reverse transcriptase-polymerase chain reaction. *Appl Environ Microbiol* **59**:3488-3491.

164. **van den Bogaard, A. E., R. Willems, N. London, J. Top, and E. E. Stobberingh.** 2002. Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. *J Antimicrob Chemother* **49**:497-505.
165. **Veneri, D., A. Vantarakis, G. Komninou, and M. Papapetropoulou.** 2004. Differentiation of faecal *Escherichia coli* from human and animal sources by random amplified polymorphic DNA-PCR (RAPD-PCR). *Water Sci Technol* **50**:193-8.
166. **Walters, S. P., and K. G. Field.** 2006 (in press). Detection of *Bacteroidales* fecal indicators and the zoonotic pathogens *E. coli* O157:H7, *Salmonella*, and *Campylobacter*. *Environ Sci Technol*.
167. **Weaver, R. W., J. A. Entry, and A. K. Graves.** 2005. Numbers of fecal streptococci and *Escherichia coli* in fresh and dry cattle, horse, and sheep manure. *Can J Microbiol* **51**:847-51.
168. **Whitman, R. L., M. B. Nevers, and M. N. Byappanahalli.** 2006. Examination of the watershed-wide distribution of *Escherichia coli* along Southern Lake Michigan: an integrated approach. *Appl Environ Microbiol* **72**:7301-10.
169. **Whitman, R. L., D. A. Shively, H. Pawlik, M. B. Nevers, and M. N. Byappanahalli.** 2003. Occurrence of *Escherichia coli* and enterococci in *Cladophora* (Chlorophyta) in nearshore water and beach sand of Lake Michigan. *Appl Environ Microbiol* **69**:4714-4719.
170. **Whittam, T. S., M. L. Wolfe, and R. A. Wilson.** 1989. Genetic relationships among *Escherichia coli* isolates causing urinary tract infections in humans and animals. *Epidemiol Infect* **102**:37-46.
171. **Wiggins, B. A., R. W. Andrews, R. A. Conway, C. L. Corr, E. J. Dobratz, D. P. Dougherty, J. R. Eppard, S. R. Knupp, M. C. Limjoco, J. M. Mettenburg, J. M. Rinehardt, J. Sonsino, R. L. Torrijos, and M. E. Zimmerman.** 1999. Use of antibiotic resistance analysis to identify nonpoint sources of fecal pollution. *Appl. Environ. Microbiol.* **65**:3483-3486.
172. **Wiggins, B. A., P. W. Cash, W. S. Creamer, S. E. Dart, P. P. Garcia, T. M. Gerecke, J. Han, B. L. Henry, K. B. Hoover, E. L. Johnson, K. C. Jones, J. G. McCarthy, J. A. McDonough, S. A. Mercer, M. J. Noto, H. Park, M. S. Phillips, S. M. Purner, B. M. Smith, E. N. Stevens, and A. K. Varner.** 2003. Use of antibiotic resistance analysis for representativeness testing of multiwatershed libraries. *Appl. Environ. Microbiol.* **69**:3399-3405.
173. **Yan, T., and M. J. Sadowsky.** 2006. Determining Sources of Fecal Bacteria in Waterways. *Environ Monit Assess* **Oct 28** [Epub ahead of print]
174. **Zhou, L., R. Fayer, J. M. Trout, U. M. Ryan, F. W. Schaefer, 3rd, and L. Xiao.** 2004. Genotypes of *Cryptosporidium* species infecting fur-bearing mammals differ from those of species infecting humans. *Appl Environ Microbiol* **70**:7574-7.
175. **Zhou, L., H. Kassa, M. L. Tischler, and L. Xiao.** 2004. Host-adapted *Cryptosporidium* spp. in Canada geese (*Branta canadensis*). *Appl Environ Microbiol* **70**:4211-5.