Microbial Source Tracking: Its Utility and Limitations Toward the Protection of Recreational Waters in the Great Lakes Basin

Summary. Microbial source tracking is necessary because standard methods of measuring fecal contamination in water by enumerating fecal indicator bacteria (FIB) do not identify the source or sources of the contamination. Source tracking methods can be divided according to whether or not they require a library (a "host origin database" or set of bacterial isolates from fecal samples of known origin). Methods can also be divided by whether or not they use FIB and by whether or not they require cultivation of microbes. These variables all affect the cost, time required, and effectiveness of the methods.

Prominent library-dependent methods include antibiotic resistance analysis (phenotypic) and fingerprinting analyses (genotypic) such as ribotyping, REP-PCR and PFGE. Library-depend methods are costly, time consuming, and have complex and sometimes poorly understood requirements for sample sizes and analyses. In addition, new libraries are needed for each geographical region. Prominent non-library-dependent methods include chemical markers (e.g. fecal sterols/stanols, caffeine and laundry whiteners) and host-specific PCR (e.g. of of *Bacteroidales* molecular markers). Another set of techniques involves direct molecular monitoring of human viruses such as adenoviruses and enteroviruses. Viruses are important because they are not well correlated with FIB but are important pathogens.

All of these methods have been tested at the proof-of-concept level, but there have been few organized comparisons and proficiency tests with blind samples. In one such study, the SCCWRP study, host-specific PCR performed well, as did ribotyping and PFGE. Other comparative studies have found somewhat different results. All support the following conclusions: 1) No method is quantitative; 2) Results from the same method differ depending on the operator and on differences in experimental design and analysis. Few studies have followed up the results of fecal source tracking to quantify resulting gains in water quality.

The best evidence supports taking a multi-tiered approach to source tracking, moving from general to specific and from less to more expensive. The first step is intensive surveys using FIB, to target sources spatially and temporally. Once "hot spots" are identified, then very directed source tracking can be done if needed, starting with less-expensive methods that identify human contamination, and continuing to more-expensive ones as needed, to identify common species, or finally to identify all species.

Companies that offer source-tracking services should be provided with blind proficiency samples to assess their abilities and estimate possible benefits, *before* they are hired.

Water quality standards were established based on epidemiological studies that measured human health outcomes following recreational exposure to human-derived fecal contamination. There are no similar studies for exposure to animal fecal contamination, although it is logical to assume that the risk from animal fecal contamination is lower. Thus even if microbial source tracking shows that fecal contamination is animal-derived, there is usually no way to allow for a higher permitted level of FIB. Hence the benefits from microbial source tracking at the present time are only that it allows the source or sources of fecal contamination to be accurately assigned, located, and corrected. In some cases this could lead to a reduction in FIB. In other cases where the source is primarily wildlife and there is no way to control the wildlife, no immediate water quality benefit from microbial source tracking will be seen. National environmental health agencies must take the responsibility to fund the required epidemiological studies so microbial source tracking can be properly applied to estimate human health risk.

Field, K. G. 2006. Microbial Source Tracking: Its Utility and Limitations Toward the Protection of Recreational Waters in the Great Lakes Basin. U.S.-Canada International Joint Commission Science Advisory Board.

Microbial Source Tracking: Its Utility and Limitations Toward the Protection of Recreational Waters in the Great Lakes Basin

Introduction

The problem: Fecal contamination of surface waters is widespread in the United States, Canada and worldwide. The resulting illnesses, beach closures, environmental and habitat degradation, and contamination of fisheries have broad economic, health and environmental impacts. The Great Lakes Basin, a major recreational site, presents many opportunities for human exposure to contaminated waters. Some of the important related issues in the Great Lakes Basin include beach closures, combined or sanitary sewer overflows, failing septic systems, agricultural and storm water runoff, pets at beaches, low water levels, extensive wildlife populations including Canada geese and gulls at beaches, fish kills, and algal blooms. Bacterial water quality standard exceedances have occurred at sites in all five Great Lakes and throughout the Basin.

Several severe recent waterborne disease outbreaks have underscored the importance of the problem of fecal contamination. The 1993 outbreak of cryptosporidiosis in Milwaukee, Wisconsin, is estimated to have affected 400,000 people (74) at an estimated cost of 96 million dollars US (21). In 2000 in Walkerton, Ontario, 2,300 people became ill and 7 died as a result of drinking water contaminated with *E. coli* O157:H7 from cow manure (56).

Direct monitoring for pathogens. Since the public health concern is microbial disease, the most straightforward approach to protecting health would be to directly monitor microbial pathogens in water. However, although effective assays for many pathogens exist, currently these are often expensive, time consuming and technically complicated. Pathogens may be rare, difficult to culture, and irregularly distributed, yet highly infectious even at low doses. Furthermore, a large number of assays for different pathogens would be required, and feces from both humans and animals may contain as-yet-unidentified pathogens or pathogens for which no assays exist. While microarrays to simultaneously assay numerous pathogens are under development (e.g. see (7, 69, 77, 119, 120), these still have numerous problems with sensitivity, specificity, and quantitation; it is likely to be a while before they are ready for general use.

Fecal indicator bacteria. Because of these limits to direct monitoring of pathogens, it is standard practice to monitor fecal indicator bacteria (FIB) such as total and fecal coliforms, *Escherichia coli,* and fecal enterococci, in water. FIB are easy-to-culture aerobic bacteria. Their presence in water is assumed to be due to fecal contamination; they are enumerated in water samples in order to quantitatively estimate the amount of the contamination.

Epidemiological studies have established human health standards based on exposure to FIB, and associated disease, in drinking, recreational and shellfish waters (reviewed in (124)). Because the most serious threat to human health is thought to come from human, not animal, fecal contamination, these epidemiological studies took place at sites where the principal source of fecal contamination was human or human sewage. The reasoning was that this would best protect human health, by avoiding setting bacterial standards too low.

Although the use of FIB to assess water quality has unequivocally reduced human health risk, the current FIB approaches fall short in several areas.

Indicator bacteria don't identify the source of contamination. To manage water quality, the source of fecal contamination must be known, both to find and mitigate the problem, and to

estimate human health risk. A variety of warm-blooded, and even some cold-blooded, animals contain FIB in their feces (e.g., see (50)). Thus, the presence of indicator species in water is not sufficient to determine the source of fecal contamination; standard methods of measuring fecal contamination by growing public health indicator bacteria do not identify the source of the contamination.

Relative risks from human and animal fecal contamination. Indicator counts lump together many different potential sources of fecal contamination, which may have wholly different associated pathogens. Human and animal feces both pose threats to human health. The health threat from human fecal contamination is well documented. For example, human feces are commonly associated with the spread of *Salmonella enterica* serovar typhi, *Shigella* spp., hepatitis A virus, and noroviruses. Indeed, until recently, the focus of concern for water-related illness, and associated research, has been contamination by human effluent (reviewed in (122)).

The human risk from domestic and agricultural animal feces is usually assumed to be less than the risk from human feces, in part because viruses, which are the most common cause of human illnesses from exposure to fecal contamination in water, are highly host-specific. Domestic/agricultural animals spread many pathogens, including, for example, *Salmonella, E. coli* O157:H7, *Campylobacter jejuni, Giardia spp., Cryptosporidium spp.*, and hepatitis E virus (reviewed in (22). Nevertheless, *few studies have been carried out on the risk of animal feces as a source of waterborne zoonotic infections* (122). In a Hong Kong study, illness rates for two marine beaches impacted by animal (pig) wastes were lower than for seven other beaches (20, 54). In a New Zealand study carried out at seven populous marine beaches, no substantial differences in illness risks were found between the human and animal waste-impacted beaches, though both were markedly different from the control beaches (78).

The disease risk from fecal contamination of wild animals, such as gulls, is poorly understood. Rare events when wild animal viruses cross into humans may be deadly; HIV/AIDS and H1N5 bird flu are prominent examples. Certain waterborne bacterial and protozoan pathogens of wild animals have been documented to infect humans (e.g. *Leptospira interrogans). Giardia* and *Cryptosporidium* widely infect wild animals. Because these parasites appear morphometrically identical in animals and humans, wild animals have long been assumed to be reservoirs and important sources of human infection. However, molecular evidence has made it clear that most genotypes of these parasites are host-adapted and cannot cross-infect among different host species (e.g. see (4, 130)). For example, in Canada geese, Zhou and colleagues concluded that "Canada geese might only serve as accidental carriers of cryptosporidia infectious to humans and probably play a minor role in the animal-to-human transmission cycle of the pathogen" (131). However, a significant number of emerging and re-emerging waterborne zoonotic pathogens have been recognized (9). Some of these pathogens may not be of recent origin; some may have been causing illness for a long time, but were not previously identified due to a lack of suitable isolation and identification methods. These include, for example, vesiviruses, *Campylobacter jejuni, Yersinia enterocolitica*, and *Cryptosporidium spp.* (94, 114, 122).

Amounts of indicator bacteria in different types of feces. Water regulators frequently must estimate total maximum daily loading (TMDLs) of fecal bacteria based on indicator counts. Although the data are sometimes contradictory (e.g. see (1, 29, 32, 33, 40, 125), there seems to be agreement that different species contain both different numbers and different relative proportions of *E. coli* and enterococci in their feces, making it unclear how to estimate the *E. coli* or FIB contribution of different sources of feces in a watershed, even if the amount of fecal input from each is somehow known.

Environmental survival of indicator bacteria. An adequate fecal indicator should not reproduce outside the animal host. Both *E. coli* and enterococci can survive and persist ubiquitously in natural environments such as fresh water lakes and streams, algal wrack, beach sand, and tropical soils (16, 37, 91, 100, 126).

Correlation of indicator bacteria with pathogens. An indicator should be correlated with the presence of pathogens; and it should have a survival profile similar to the survival profile of the pathogens whose presence it indicates. *E. coli* and enterococci are not well correlated with pathogenic *Salmonella* spp. (68), *Campylobacter* spp. (10, 55, 68, 72), *Cryptosporidium* spp. (10, 55, 68, 72), human enteroviruses (39, 55, 68), including adenoviruses (87), and coliphage (60). The poor correlation of bacterial indicators with viruses is of particular concern because of the low infectious dose of the viruses (36), their linkage with both acute and chronic disease (36), and the fact that they are considered the most frequent cause of swimmer-associated illnesses.

Microbial Source Tracking. Diagnosing the sources of fecal contamination in water is typically called bacterial source tracking (BST) or microbial source tracking (MST). These names are misleading, as they imply that microbes or bacteria don't normally occur in water and only come from fecal contamination. However, *naturally* occurring microbes are ubiquitous in surface waters, with bacteria occurring at an average density of 10^6 cells/ml and viruses at a higher density.

The assumptions that underlie fecal source tracking are that some characteristic in feces unequivocally identifies a particular feces type; and that this identifying trait can be detected in water. Furthermore, many methods make the (usually untested) assumption that the relative proportion of identifying traits remains the same in water over time as the relative proportion in the original feces that entered the water; therefore, if the traits can be quantitatively detected, the quantitative contribution of each particular type of feces can be estimated.

As an example, an earlier method to distinguish human from non-human fecal contamination was based on estimating the ratio of fecal streptococci to fecal coliforms. But because strains of coliforms and streptococci have different survival rates, the ratio changes in complex ways over time, making it unreliable (112, 113).

There are several recent reviews of fecal source tracking (29, 80, 108, 111).

Why Microbial Source Tracking? There may be several reasons to do microbial source tracking. The first concern is frequently to *investigate the source of high levels of FIB*. From the point of view of regulators, identifying the source or sources of FIB (not feces, not pathogens) and eliminating them may be the *only* objective. A second objective is to identify particular pathogens in water. Certain sources of fecal pollution might be associated with particular pathogens (for example, *E. coli* O157:H7 with ruminant feces). This reason is closely related to the third reason, which is to estimate the human health risk associated with exposure to contaminated water.

The first objective is obtainable; the others may not be.

Microbial Source Tracking: Methods

A number of methods for fecal source identification are in use or under development. These can be divided into culture-based and culture-independent methods. Furthermore, some methods require a "library". In this context, a "library" is a set of bacterial isolates from fecal samples of known origin, tested using the method of source discrimination. It is also called a "host origin

database." Most library methods are culture-based, and require growing environmental isolates from water samples. Source identification occurs by a statistical 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 microbial (molecular) tests.

Culture-based, library-dependent methods.

It is logical to base fecal source identification methods on FIB, because throughout the US and Canada, FIB are used to identify a water quality problem in the first place. Moreover, epidemiological studies correlating FIB with health risks have already been done, and water quality laboratories are expert at indicator assays. Thus library-based methods have typically started by growing *E. coli* or enterococci.

Library-based approaches are labor-intensive, requiring extensive sampling both to prepare the library and to test environmental isolates. All library-based methods have complex requirements for adequate sample size, representativeness, and geographic stability (52, 83, 129). Data on geographic stability suggest that for most methods, libraries are not cosmopolitan, and thus a separate library for each locale or watershed may be required (e.g., see (48, 95, 110, 115)).

Antibiotic resistance and other phenotypic methods. In the multiple antibiotic resistance (MAR) method, also called ARA (antibiotic resistance analysis) and ARP (antibiotic resistance profiling), isolates of *E. coli* or enterococci are tested against panels of antibiotics in order to discriminate among human and various animal sources of fecal pollution (e.g. see (26, 47, 51, 52, 96, 128)). The underlying assumption is that since humans, agricultural animals, and wildlife have been exposed to different antibiotic regimes, their fecal bacteria will differ in types and levels of antibiotic resistance.

Antibiotic resistance traits in bacteria are often borne on plasmids, are under strong selection, and change rapidly under the influence of host population exposure to antibiotics and other chemicals. Thus, antibiotic resistance is not geographically stable; libraries of strains from known sources must be constructed for each new geographic region being tested. However, the MAR/ARA method is inexpensive and low-tech, making it readily available to a broad variety of investigators.

Comparative studies that have assessed the effectiveness of antibiotic resistance methods for fecal source tracking have generally given them low ratings (e.g. see (52, 82)). In a blind study that compared a number of fecal source tracking methods using water samples containing feces (see below), the performance of antibiotic resistance-based methods at identifying the fecal sources was little different from random (45). There is some evidence that enterococci work better than *E. coli* for ARA (see (52, 82)).

Other phenotypic methods that have been tested include carbon-source utilization (CUP) and serotyping; the serotyping approach did not seem promising and was not extensively tested, and CUP did not perform well in a comparative study (45).

DNA fingerprinting: ribotyping, REP-PCR, and related methods. Genotypic library-based methods are usually based on DNA fingerprinting of bacterial isolates. Ribotyping, repetitive extragenic palindromic polymerase chain reaction (REP-PCR), amplified fragment length polymorphism (AFLP), pulsed-field gel electrophoresis (PFGE), random amplified DNA

polymorphisms (RAPD), and denaturing gradient gel electrophoresis (DGGE) are fingerprinting techniques producing bar code-like patterns for each bacterial isolate.

Each of the fingerprinting methods depends on matching the fingerprint patterns of bacteria isolates from known sources of feces (the library) with fingerprint patterns from individual water isolates. Each fingerprinting assay is complex and different, requiring specialized equipment and training. For example, ribotyping involves growing indicator bacterial isolates, extracting DNA, amplifying 16S rRNA genes by PCR, and digesting amplification products with restriction enzymes. The "bar code" is usually created by separating fragments via electrophoresis, and detecting fragment patterns by hybridization with radioactive or fluorescent probes (e.g., see (19, 48, 83, 97, 105)). The resulting patterns can be very small, complex, and difficult to distinguish and interpret, either by eye or automatically.

For these methods, the size of the "library" is extremely important, as is the method of statistical analysis used (53, 61, 118). In addition, many studies have found that many or most environmental isolates cannot be matched to host isolates (e.g. see (53, 118). It is necessary to discard these unmatched isolates; identifying them based on similarity to known isolates results in incorrect classifications.

Ribotyping and PFGE are successfully used for epidemiology of food outbreaks, to identify an outbreak and its source. However, source-tracking presents considerably more complex problems than matching outbreak genotypes. In a blind study that compared a number of fecal source tracking methods using water samples containing feces (see below), the performance of both ribotyping and PFGE was good (45, 83); REP-PCR was not as good, and the other fingerprinting methods were not tested. Several investigators have concluded that PFGE may be of too great resolution for source tracking (e.g. see (71)); however, commercial source tracking companies use it. A more recent comparison between ribotyping and antibiotic resistance profiling found that ribotyping only identified 27% of unknowns to the correct source category; *E. coli* ARA correctly identified 28% and enterococci ARA correctly identified 45%.

Other genotypic library-based methods Ram and colleagues explored directly sequencing beta-glucoronidase genes from *E. coli* isolates, and comparing sequences to library sequences (103). They found 114 alleles (different sequences) in environmental isolates; different allele frequencies occurred at different sites. Of 82 alleles from fecal samples, a few were host-specific (2 in birds, 3 in humans), but the most common alleles were found in all of the hosts. Their "internal consistency" (ability to correctly assign isolates) was 60 to 75%.

Library-independent, culture-dependent methods.

Viral methods. Phage of *Bacteroides fragilis* can distinguish human and animal fecal pollution, as certain strains of *B. fragilis* will grow bacteriophages only from human sewage and others will support phage growth from both human and animal feces (102). However, these phages are more common in Europe, particularly southern Europe, and may not be useful in the US and Canada (e.g. see (98)). Similarly, two serotypes of F+ RNA coliphages, Types II and III, are found in association with human fecal contamination, whereas Type IV is found in association with animal fecal contamination and Type I occurs in both human and animal feces (38, 49). Growth of these coliphages in an appropriate cell culture, followed by serotyping, identifies human and non-human fecal contamination in water. Recently, serotyping has largely been replaced by molecular typing (57).

These viral methods are limited to discriminating between human and animal sources. Little is known about differential survival of the various types, which would affect the ability to

discriminate over time. In addition, the markers appear to be irregularly distributed in populations and may work better in some geographic areas and when fecal sources comprise multiple individuals (such as from sewage) rather than single individuals (86). Culture-based viral detection methods are largely being replaced by molecular detection (see below).

Bacterial methods. Several microbial source tracking methods are based on culturing host-specific bacterial strains, such as *Bifidobacterium adolescentis* for humans (11, 73, 104) and *Rhodococcus coprophilis* for grazing animals (92, 107). They are isolated and detected with selective media and colony hybridization; molecular detection is coming into widespread use.

A recent revival of the ratio approach suggests that the bacterial ratio of atypical colonies to total coliform colonies (AC/TC) from a total coliform membrane filter assay could identify human fecal and agricultural impacts (12).

Culture-independent, library-dependent methods

Community fingerprinting. This approach uses T-RFLP, a technique that digests community DNA with restriction enzymes, then separates fluorescently labeled fragments according to size on a DNA automated sequencer. The pattern of fragments is then compared to patterns generated from known fecal DNAs. These known fecal patterns make up the "host origin database"; like a library of bacterial isolates, they are unlikely to be geographically or temporally stable and likely to need to be re-created with relevant fecal samples for each new study. As well as demanding a DNA automated sequencer, equipment that a water quality lab would be unlikely to own, this technique did not perform well in a blind study that compared a number of fecal source tracking methods using water samples containing feces (see below) (30, 45).

A similar approach used cluster analysis to compare T-RFLP patterns from *Bacteroides-Prevotella* fecal DNAs derived from a number of species including chickens, cows, deer, pigs, dogs, geese, horses, humans, and gulls (34). The results were not promising. Using fecal DNAs, there was great overlap among species, and although cluster analyses separated the patterns obtained from single species, no species-specific diagnostic peaks were found. Even if a laboratory was equipped to carry out this analysis, it is unlikely that the technique could identify fecal sources mixed together in environmental samples.

Culture-independent, library-independent methods

Chemical methods. Not being based on microbes, chemical methods are both "culture-independent" and "library-independent". Substances such as caffeine, fecal sterols and stanols, laundry brighteners, surfactants, fragrances, pesticides, and polycyclic aromatic hydrocarbons can be used to detect human and non-human fecal contamination and determine urban sources of fecal contamination (15, 27, 58, 67, 85, 101, 113, 116, 121). Standley and colleagues (116) compared several of these so-called "molecular tracers" and concluded that a combined index of caffeine and fragrance levels was an effective identifier for human sewage; a ratio between particular steroids made an effective identifier for agricultural input; and a different steroid ratio identified wildlife input. Similarly, profiling of seven sterols in South Australian metropolitan catchments suggested areas of human, dog, and bird fecal impact (121).

Although presence of chemical indicators and molecular tracers can identify recent fecal inputs, their spread, transport, and persistence in water may not be correlated with that of pathogens and FIB, which are cellular or particulate. Survival of indicators and pathogens in water is affected by factors such as settling, UV irradiation, and grazing (e.g. see (14)); these factors are likely to affect chemical indicators differently than cellular or particulate pathogens.

These methods have been tested in Australia and are in widespread use there. A recent study compared a suite of fecal steroids from the Santa Ana River in California and found that the steroid ratios were inconsistent with sewage; moreover, concentrations of FIB were correlated with occurrence of bird fecal steroids (90).

Molecular methods. This approach represents a large change in water quality monitoring, as in most cases the methods not only avoid culturing, but also may not use standard fecal indicators at all. 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. These methods assay specific genes by the polymerase chain reaction (PCR); 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 grow. These methods can theoretically take as little as two or three hours from sampling to diagnosis.

PCR, a method of making many copies of a specific DNA sequence in a test tube, is routinely used in medical and food diagnostic labs, and has replaced many older diagnostic procedures that took weeks and required multiple differential media and biochemical tests. Thus although many water labs are not yet equipped for PCR, the technique is accessible, the equipment is not expensive (comparable to, say, an incubator, and far less than a centrifuge) and the technology is likely to be increasingly available.

The assumption that underlies this approach is that there are host-specific genetic markers in feces. These may be markers that are human-specific, to separate human from non-human fecal pollution, or markers that specifically identify individual host species. Although a single individual's fecal community may change over time and in relation to diet and age (although recent molecular data are challenging this assumption, see (76)), certain features persist and are diagnostic. However, host-specific markers may not be present in every individual, and individuals may have differing amounts of the markers. As a result, these methods usually work better when there is a "bulk" or community sample (such as sewage, for humans) rather than an individual or family sample.

Viral methods. A number of fecal viruses can be monitored directly in water, without culturing (reviewed in (35, 43). Examples include human adenoviruses, human enteroviruses, and bovine enteroviruses (36, 44, 59, 70, 88, 99, 123). The presence of human or bovine viruses indicates the presence of human or cattle fecal pollution. Monitoring for viruses typically requires larger water samples than the 100 ml samples used for water quality monitoring; concentration of such large samples can concentrate PCR-inhibitory substances as well, interfering with detection (59, 119). To increase sensitivity, investigators may use nested PCR, which makes it difficult or impossible to detect quantitatively (59). Real-time PCR assays have successfully quantified enteric viruses (8, 81). The viral methods are effective in detecting human sewage, although they may not detect feces from individuals or small groups of humans (86). These methods are particularly important because they directly detect viral pathogens, which are not well correlated with FIB.

Anaerobic bacterial targets Some fecal anaerobic bacteria (for example, in the genera *Bifidobacterium* and *Bacteroides*) have host-specific distributions and can therefore identify particular sources of fecal contamination (e.g. see (2, 31, 64, 104). Fecal anaerobes make up the majority of bacterial cells in feces, and are present at much higher densities than coliforms and enterococci. However, because culturing anaerobes is far more complex that growing FIB, anaerobes were not generally adopted as indicators until a shift to molecular rather than culture-based methods for studying bacteria in natural populations spilled over into the public health arena.

This shift to molecular ecology of microbes led to the discovery that the vast majority of bacteria in all habitats, ranging from soil and water to the mouth and GI tract, have never been grown, and indeed cannot be cultivated using standard approaches. The importance of this discovery for source tracking is that "uncultivated" bacteria provide potential host-specific molecular markers. In fact, because the majority of bacteria in feces have never been grown, "uncultivated" targets are more common in feces that cultivated ones.

Ribosomal RNA genes are commonly used for molecular diversity studies of uncultivated bacteria, because these genes are present in all bacterial groups, allowing the use of published PCR primers; in addition, these genes contain sufficient sequence diversity to distinguish among specific strains, species or genera. Most bacterial groups have multiple rRNA gene copies, increasing the ease of detection. Other good targets are rRNA gene spacers.

Bacteroides and related genera (Phylum and Class *Bacteroidetes*, Order *Bacteroidales*) comprise a large proportion of the fecal flora in warm-blooded animals, making them a relatively easy target for detection. In addition, they are genetically diverse, are limited to animal body cavities, are unlikely to survive long after release into receiving waters (because they are anaerobes), and show species- or group-specific host distributions (2, 31, 64). *Bacteroidales* host specific PCR primers based on uncultivated microbes can specifically identify feces from ruminants, humans, dogs, pigs, horses and elk (5, 23). Markers can be detected in fecally contaminated natural waters. Limits of detection of host-specific markers in 100 ml water samples is comparable to the limits of detection of *E. coli* by culture ((5, 6, 13, 24). These assays appear to be geographically stable, and have been used successfully throughout the United States and Canada, northern Europe, Hawaii and New Zealand.

In a blind study that compared a number of fecal source tracking methods using water samples containing feces (see below), host-specific PCR of *Bacteroidales* molecular markers performed well.

However, because there are many closely related *Bacteroidales* sequences in feces along with the host-specific sequences, and because even identical models of thermal cyclers vary significantly, it is important for each user to establish the specificity of the host-specific assays by testing them with fecal DNAs from the target species and other species. PCR conditions can usually be adjusted so that detection is specific.

Nor only do related hosts have closely-related fecal *Bacteroidales* (for example, ruminants), suggesting co-evolution and adaptation, but horizontal transfer of fecal bacteria among species in close contact has also occurred (e.g. humans and their pets; (23)). Sooner or later, therefore, it's likely that someone will find dogs, chickens or gulls that bear the published "human specific" markers, and so on. It is important to test host-specific primers each time they are used in a new locale.

A PCR assay for *B. thetaiotaomicron* distinguishes human and dog feces from other animals (18).

Similar approaches have targeted the genus *Bifidobacterium*. Nebra and co-workers (84) developed probes to distinguish *B. dentium*, a species thought to be limited to humans, and animal-specific *Bifidobacterium* species. Following amplification of *Bifidobacterium* 16S rDNAs using general primers, they used their probes to differentiate human and animal samples. There is some concern about survival of this group in water. Resnick and Levin (104) found that members of the genus *Bifidobacterium* could not be cultured after 5 h in fresh water or 10 h in salt water. Carillo and colleagues (17) also observed very low survival of *B. adolescentis* in a

tropical environment and suggested that the genus could be used to detect only very recent fecal contamination. These cultivability problems may not matter for molecular detection.

Toxin/virulence genes. Host-specific toxin genes in *E. coli* make interesting targets for source detection, as they are not only related to standard fecal indicators, but give information about pathogen status. A heat-stable enterotoxin from enterotoxigenic *E. coli*, the STIb toxin, is associated with human fecal waste; its gene is the target for PCR primers that detect human fecal pollution (93). Similarly, the STII toxin gene is associated with pig feces; specific primers can detect pig fecal contamination (63). The heat-labile enterotoxin, LTIIa, is associated with cattle fecal waste; its gene is the target of PCR primers to detect fecal pollution from cattle (62). These markers are generally specific (with occasional exceptions (30)), and are temporally and geographically stable. One drawback is that the target genes are relatively rare. In a blind study that compared a number of fecal source tracking methods using water samples containing feces (see below), host-specific PCR of toxin genes performed well, but it was necessary to enrich for *E. coli* from the 100-ml water samples before the toxin genes could be detected. This precludes quantitative detection, as the growth step introduces culture bias. Thus the method is not truly culture-independent, and will take longer than methods that directly sample genes in water without an intervening growth step.

Similarly, a virulence factor from *Enterococcus faecium*, the enterococcal surface protein (ent) is the target for a human-specific PCR assay (109). This assay is highly specific; 97% of human sewage and septage samples, but no livestock or bird samples, were positive for the marker. However, the assay is not very sensitive. Like the *E. coli* toxin genes, the *ent* gene is rare; it is necessary to enrich for enterococci before the gene can be detected, precluding quantitative detection and increasing the time required.

Rhodococcus coprophilus. Culture-based detection of this bacterial indicator of fecal contamination from herbivores (cow, sheep, horse and deer) is slow. Detection by PCR and quantification by Q-PCR is specific and sensitive (107).

Host mitochondrial sequences. Martellini and colleagues developed PCR primers targeting host mitochondrial gene sequences (75). It is well known that hosts shed their own cells (e.g. blood cells, intestinal cells) in feces. It seems incontrovertible that these host cells make a better host-specific target for source tracking than bacteria, which may be found in multiple hosts and can spread among species. Mitochondrial DNA is more common than nuclear DNA, and is well known to be more variable. This approach appears extremely promising if the bugs can be worked out. The initial publication showed many unresolved problems with specificity and sensitivity.

Culture-independent Versus Culture-Dependent Methods

Advantages and disadvantages of culture-based methods. Culturing fecal indicator organisms is relatively inexpensive and low-tech, making it broadly available. However, this advantage is lost if the source identification method that is applied to the cultured isolates is high-tech and expensive (e.g., PFGE and AFLP). Another advantage of culturing is that it provides an enrichment step, increasing the numbers of target microorganisms and providing single strains in isolation. Finally, culture-based methods often use standard public health indicators such as *E. coli* or enterococci, for which at least some information about survival and transport is available.

Disadvantages are that these methods are limited to testing easily cultured microbes. Many pathogens, and even the most common fecal bacteria, are difficult to grow. In addition, the

composition of microbial communities changes drastically when cultured (e.g., see (28)). This "culture bias" has virtually never been considered in culture-based fecal source identification.

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 may instead use difficult-to-grow but common fecal microbes or mine the uncultured genetic diversity in feces for source-specific markers.

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, any other markers must be correlated with public health bacteria in order for managers to use them.

PCR carries high risks of contamination. As a result, another disadvantage of these methods is the necessity of establishing stringent controls at all steps of the process, from physical separation of different stages of the research into different laboratories to inclusion of appropriate negative controls.

A further limitation of the culture-independent methods is that markers for only a few animal species are currently available; wildlife species especially are not represented. More and different gene targets are needed. Most of the culture-independent methods result in presence/absence data on marker occurrence; quantitative assays are needed. Finally, for any of these markers, it is important to test their geographic range and temporal variability.

Microbial Source Tracking Using Combined Methods

As no single source tracking method is ideal, some have suggested combining methods (45, 117), in order to enhance discrimination or provide confirmation of results. Boehm and colleagues used intensive testing for FIB to spatially locate areas of intense contamination and characterize variability in Catalina Bay (8). They followed this with targeted assays for human-specific *Bacteroidales* and enteroviruses to identify the source of the contamination. They concluded that there were multiple sources; the spatial component of their sampling allowed them to identify specific sources, including a leaking graywater pipe.

In a second study, intensive FIB sampling in a watershed emptying into Santa Monica Bay was combined with molecular detection of enterovirus, a human-specific *Bacteroidales* marker, and enterococcus. Finally, investigators sequenced amplified enterovirus sequences to confirm the presence of potential risks to human health. Although the entire creek had high FIB levels, high human-specific and viral indicators in specific areas indicated where mitigation would do the most good (89).

Genthner and colleagues combined REP-PCR fingerprinting with antibiotic resistance analysis of *Enterococcus faecalis* isolates, and concluded that the combination of the two increased their ability to assign beach/swash zone isolates as either human or gull-derived. Most isolates were identified as gull. It is striking that in this study there were no matches among isolates. Cluster analysis of REP-PCR patterns placed human, gull, and beach isolates in separate lineages; ARA also clustered beach isolates in a discrete lineage, with gull and human isolates intermingled in lineages. This study appears to support the existence of unique environmental lineages, rather than identifying them as human or gull-derived.

In New Zealand investigators combined identification of host-specific *Bifidobacterium*, *Rhodococcus* and *Bacteroides* with assays for fluorescent whitening agents and fecal sterols/stanols. They were able to identify human contamination, but found that animal input was more difficult (41).

Microbial Source Tracking: Is it Quantitative?

Because FIB are used quantitatively to estimate total fecal load, water quality practitioners accept without question, and in fact insist, that fecal source tracking methods should be quantitative. However, little is known about the comparative survival of the different kinds of source-specific markers, and what data there are indicate strongly that survival is not proportional. Many different studies have shown that populations of *E. coli* in fresh feces differ from strains sampled from diverse habitats such as dry feces, animal bedding, septic tanks, storage lagoons, and water samples (e.g. see (42, 79, 127). The general trend in the environment outside the host, confirmed by several different measures of genetic variability, is dominance of environmental strains that differ from strains in the host. A study that used ribotyping to follow persistence and differential survival of *E. coli* genotypes, for example, showed that some strains were more persistent than others, and that the distribution of ribotypes in environmental mesocosms was different from their distribution in feces (3). Another study of diversity of *E. coli* in the environment versus in feces found that rivers and beaches were dominated by river and beach genotypes, which differed from fecal genotypes even when the environments in question were heavily fecally contaminated (79).

Under these circumstances, it is hard to imagine how fecal source tracking could be more than vaguely quantitative. If the proportions of the markers change as soon as they hit the water, and if the markers all show differential survival, and if fecal bacterial genotypes in water are dominated by unique environmentally-adapted strains, then trying to make exact quantitative estimates of the contribution of different fecal sources doesn't make sense. This is particularly true for culture-based assays, where selection of readily cultivable strains leads to bias. However, used as presence-absence tests, microbial source tracking is useful both to identify fecal sources and to locate "hot spots" of contamination.

In a blind study that compared a number of fecal source tracking methods using water samples containing feces (see below), all methods failed to quantify fecal inputs in unknown samples (45).

Microbial Source Tracking: How Should the Methods be Assessed?

The field of microbial source tracking is still in early development in some ways. Many of the methods have only been tested against fecal samples in laboratory studies (proof of concept testing), or applied in field studies where the "real" answer is not known, so the real performance of the method cannot be assessed. Two kinds of testing are needed. The first is blind testing with proficiency samples; this could be done comparatively to rank methods and to better establish relative costs and strengths of each. The second is application of the source-tracking method in field studies, followed by measurement of the resulting improvement in water quality.

ARCC and other statistical tests. The average rate of correct classification (ARCC) has been used to judge how well library-based source tracking methods work. ARCC is a statistical estimate of the ability of a library to correctly classify isolates pulled from the library (*not* its ability to correctly classify environmental isolates or known-source isolates from outside the library). ARCCs reported in some studies have been quite high (e.g., see (25, 46)). However,

the size of the library influences its ARCC. Small libraries have higher ARCCs than large libraries, but small libraries are not as representative and are therefore not as good at classifying novel isolates (from outside the library) as are large libraries (82, 129). Thus, ARCC may be better termed *internal* accuracy (82). It *does not estimate the ability of the method to identify fecal sources, and can be misleading*. Because many methods of fecal source tracking have been assessed only by calculating ARCCs, the ability to compare these methods is limited.

Comparative and Proficiency Studies. An ideal test of methods would supply practitioners with blind samples for source identification. The Southern California Coastal Water Research Project (SCCWRP) and the US EPA sponsored such a study in 2003 (30, 45, 52, 83, 86). Study participants were asked to identify the fecal source(s) in identical sets of water samples containing human, cattle, dog, or gull feces, sewage, or a mixture. Along with unknown water samples, participants were supplied with samples of the feces used to create the unknowns. Study participants used coliphage and virus-based approaches, antibiotic resistance, carbon utilization profiling, ribotyping, REP-PCR, PFGE, community DNA profiling, and host-specific PCR of *E. coli* toxin genes and *Bacteroidales* molecular markers. 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 both freshwater and saltwater samples and samples with humic acids.

Host-specific PCR (of *E. coli* toxin genes and *Bacteroidales* markers) was very accurate at identifying samples with human feces and sewage with no false positives, and was generally considered to perform the best of the methods. Ribotyping and PFGE also performed well, although results varied depending on what group did the analysis. Several of the others, including phenotypic methods and genotypic library-based methods, identified most or all samples with human input, but had 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 host-specific PCR methods accurately identified the species for which they had markers, but did not have markers for all species. Many of the other methods had significant numbers of false positives. Several broad conclusions could be reached. First, the same approach did not perform equally well in the hands of different investigators, underlining the need for standardization. Second, the rate of false positives for culture-based, library-dependent methods was often very high. Third, no method was able to accurately quantify the sources. Fourth, each method had strengths and weaknesses, and no method performed perfectly. Methods that accurately identify human fecal contamination are useful when the principal question is the identification of human input. Methods that are rapid and accurate for some sources, but don't identify all sources, would be useful where the principal research objective is to identify the major sources of fecal contamination, for rapid mitigation. Methods that are more time-consuming and less accurate, but identify all sources, would be appropriate where it was important to know all sources.

A second smaller study compared library-dependent methods using *E. coli* (118). Again, ribotyping and PFGE worked well. It was notable that ribotyping with *EcoRI* and *PvuII* approached 100% correct classification of unknown isolates, but only 6% of the isolates could be classified. This is the same pattern seen in studies cited above that compared genotypes of fecal and environmental strains; most environmental strains ("transients") differ from fecal strains. If someone doing microbial source tracking with a library-based genotypic method claims to be able to assign all the environmental isolates to host, they are either being deceptive or they don't understand the method.

A third study compared the performance of ribotyping with *Hind*III and antibiotic resistance testing (82). The study established libraries, measured internal consistency (rather low, as expected with large libraries), and used the libraries to attempt to classify blind proficiency samples. Twenty-eight per cent (by ARA) and 27% (by ribotyping) of the *E. coli* proficiency isolates were assigned to the correct source category. There was almost no overlap between isolates correctly classified by the two methods. This study concluded that "None of the methods performed well enough on the proficiency panel to be judged ready for application to environmental samples."

The difference in results reported in these and other comparative studies (106) may be due to study design and operator error, underlining the necessity of accurately establishing the correct parameters for each method.

Few or no studies have accurately measured water quality improvements that resulted from source tracking. These studies are necessary in order to analyze benefits and costs.

Microbial Source Tracking: What are the Benefits?

Water quality standards were established based on the results of epidemiological studies that measured human health outcomes following recreational exposure to human-derived fecal contamination. There are no similar studies of health outcomes following exposure to animal fecal contamination, although it is logical to assume that the risk from animal fecal contamination is lower. A recent exposure study at Mission Bay, California (report available on the SCCWRP web site) 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 and found that the primary source of the FIB at Mission Bay was non-human, most likely from water birds. These results underscore the need for larger epidemiological studies to measure human health risks from animal fecal contamination. National environmental health agencies must take the responsibility to fund the required epidemiological studies so microbial source tracking can be properly applied to estimate human health risk.

Water quality regulators are frequently in the situation where high bacterial counts are thought to be due primarily to wildlife. Even if microbial source tracking shows that fecal contamination is wholly animal-derived, current regulations do not usually allow for a higher permitted level of FIB. Hence the benefits from microbial source tracking at the present time are only that it allows the source or sources of fecal contamination to be accurately assigned, located, and corrected. In some cases this could lead to a reduction in FIB. In others, where the source is primarily wildlife and there is no way to control the wildlife, no immediate water quality benefit from microbial source tracking will be seen. However, regulators must identify and eliminate all possible fecal sources; even when there is a lot of wildlife, human sewage and septage leaks and agricultural runoff may still be identified.

Microbial Source Tracking: How Should it be Done?

The best evidence supports taking a multi-tiered approach to source tracking (e.g. see (8, 65, 66, 89), moving from general to specific and from less to more expensive. After each step, progress should be assessed before deciding to move to the next one. The first step is intensive surveys using FIB, to target sources spatially and temporally. Once "hot spots" are identified, their sources may become obvious even without any specific microbial source tracking (for example, leaky pipes or run-off from a particular farm). If not, then very directed source tracking can be done, starting with less-expensive methods that distinguish human contamination, continuing to more-expensive ones as needed, to identify common or likely targeted species,

and finally identifying all species if needed. Appropriate methods to distinguish human contamination would be chemical methods (caffeine, laundry brighteners and the like), host-specific PCR (for example, *Bacteroidales* molecular markers), and viral methods. The lowest tier of species identification could also be host-specific PCR, as it is quicker and less expensive than library-based methods and can identify common agricultural and domestic animals. The final tier, if needed, would be a library-based method to identify specific sources in more detail.

Microbial Source Tracking: Who Should Be Chosen to do it?

Since the need for microbial source tracking has arisen (in part driven by availability of methods), a number of commercial companies have started offering it as a service. Some are highly experienced; others are proposing to do it without any experience or even understanding of the issues. How should a group or municipality distinguish the two and decide who to hire?

If a municipality needs source tracking, it is important that they follow the tiered approach outlined above. Companies might want to sell them the most expensive "top tier" type of source tracking (a library-based approach that would attempt to identify all the species and perhaps quantify the input from each). This may not be needed, and should not be done unless the lower tiers have already been done.

Secondly, the municipality should provide the potential providers of source tracking services with blind proficiency samples, and assess each company's ability to correctly identify the sources of contamination in the samples (*not* to provide ARCC's). Several studies (e.g. see (45)) demonstrate how to approach providing proficiency samples. It is important to provide the same kinds of samples to all companies. It is also important to provide samples that mimic what the municipality would want analyzed; if water samples, then water samples with known sources of fecal contamination should be provided, not bacterial isolates. If a company cannot provide a level of improvement in source identification proportional to the amount it will be paid, a different approach or company should be considered.

An appropriate role for a central scientific advisory board or agency would be to either provide blind proficiency samples, or to fund a reliable laboratory that could provide sets of identical proficiency samples, thus helping municipalities make good choices.

References

1.   **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.

2.   **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.

3.   **Anderson, K. L., J. E. Whitlock, and V. J. Harwood.** 2005. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. Appl Environ Microbiol **71**.

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.   **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.

6.   **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.

7.   **Bodrossey, L., and A. Sessitsch.** 2004. Oligonucleotide microarrays in microbial diagnostics. Curr Opin Microbiol **7**:245-54.

8.   **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.

9.   **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.

10. **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.

11. **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.

12. **Booth, J., and G. M. Brion.** 2004. The utility of the AC/TC ratio for watershed management: a case study. Water Sci Technol **50**:199-203.

13. **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.

14. **Brookes, J. D., M. R. Hipsey, M. D. Burch, R. H. Regel, L. G. Linden, C. M. Ferguson, and J. P. Antenucci.** 2005. Relative value of surrogate indicators for detecting pathogens in lakes and reservoirs. Environ Sci Technol **39**.

15. **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.

16. **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**:4549-4555.

17. **Carillo, M., E. Estrada, and T. C. Hazen.** 1985. Survival and enumeration of the fecal indicators *Bifidobacterium adolescentis* and *Escherichia coli* in a tropical reain forest watershed. Appl. Environ. Microbiol. **50:**468-476.

18. **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.

19. **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.

20. **Cheung, W. H. S. (ed.).** 1988. Health effects of beach water pollution in Hong Kong, vol. 88. Vincent Blue Copy Co., Hong Kong.

21. **Corso, P. S., M. H. Kramer, K. A. Blair, D. G. Addiss, J. P. Davis, and A. C. Haddix.** 2003. Cost of illness in the 1993 waterborne Cryptosporidium outbreak, Milwaukee, Wisconsin. Emerg Infect Dis **9:**426-31.

22. **Craun, G. F., R. L. Calderon, and M. F. Craun.** 2004. Waterborne outbreaks caused by zoonotic pathogens in the USA, p. 120-135. *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.

23. **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.

24. **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.

25. **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.

26. **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.

27. **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.

28. **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.

29. **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.

30. **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.

31. **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.

32.   **Fogarty, L. R., S. K. Haack, M. J. Wolcott, and R. L. Whitman.** 2003. Abundance and characterictics of the recreational water quality indicator bacteria Escherichia coli and enterococci in gull faeces. J. Appl. Microbiol. **94:**865-878.

33.   **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 feces. J Appl Microbiol **94:**865-78.

34.   **Fogarty, L. R., and M. A. Voytek.** 2005. Comparison of bacteroides-prevotella 16S rRNA genetic markers for fecal samples from different animal species. Appl Environ Microbiol **71:**5999-6007.

35.   **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.

36.   **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.

37.   **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.

38.   **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.

39.   **Geldenhuys, 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.

40.   **Geldreich, E. E.** 1978. Bacterial populations and indicator concepts in feces, sewage, stormwater, and solid wastes. *In* G. Berg (ed.), Idicators of viruses in water and food. Ann Arbor Science Publishers, Ann Arbor.

41.   **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 indentification. Water Science and Technology **47:**39-43.

42.   **Gordon, D. M., S. Bauer, and J. R. Johnson.** 2002. The genetic structure of *Escherichia coli* populations in primary and secondary habitats. Microbiology **148:**1513-1522.

43.   **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.

44.   **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.

45.   **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.

46.   **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.

47.   **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.

48. **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.

49. **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.

50. **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.

51. **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.

52. **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.

53. **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.

54. **Holmes, P. R.** 1989. Research into health risks at bathing beaches in Hong Kong. J. Inst. Water Environ. Manage. **3:**488-495.

55. **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.

56. **Hrudey, S. E., P. Payment, P. M. Huck, R. W. Gillham, and E. J. Hrudey.** 2003. A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. Water Sci Technol **47:**7-14.

57. **Hsu, F. C., Y. S. Shieh, J. van Duin, M. J. Beekwilder, and M. D. Sobsey.** 1995. Genotyping male-specific RNA coliphages by hybridization with oligonucleotide probes. Appl Environ Microbiol **61:**3960-6.

58. **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.

59. **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.

60. **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:**179-184.

61. **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.

62. **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.

63. **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. **In press.**

64. **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.

65. **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.

66. **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.

67. **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.

68. **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.

69. **Lemarchand, K., L. Mason, and R. Brousseau.** 2004. Molecular bioogy and DNA microarray technlogy for microbial quality monitoring of water. Crit Rev Microbiol **30**:145-72.

70. **Ley, V., J. Higgins, and R. Fayer.** 2002. Bovine enteroviruses as indicators of fecal contamination. Appl Environ Microbiol **68**:3455-3461.

71. **Lu, L., M. E. Hume, K. L. Sternes, and S. D. Pillai.** 2004. Genetic diversity of Escherichia coli isolates in irrigation water and associated sediments: imlications for source tracking. Water Res **38**:3899-908.

72. **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.

73. **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.

74. **Mac Kenzie, W. R., N. J. Hoxie, M. E. Proctor, M. S. Gradus, K. A. Blair, D. E. Peterson, J. J. Kazmierzak, D. G. Addiss, K. R. Fox, J. B. Rose, and J. P. Davis.** 1994. A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. New Eng. J. Med. **331**:161-167.

75. **Martellini, A., P. Payment, and R. Villemur.** 2005. Use of eukaryotic mitochondrial DNA to differentiate human, bovine, porcine and ovine sources in fecally contaminated surface water. Water Res **39**:541-8.

76. **Matsuki, T., K. Watanabe, J. Fujimoto, T. Takada, and R. Tanaka.** 2004. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. Appl Environ Microbiol **70**:7220-8.

77. **Maynard, C., F. Berthiaume, K. Lemarchand, J. Harel, P. Payment, P. Bayardelle, L. Masson, and R. Brouseau.** 2005. Waterborne pathogen detection by use of oligonucleotide-based microrrays. Appl Environ Microbiol **71**:8548-57.

78. **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.

79. **McLellan, S. L.** 2004. Genetic diversity of *Escherichia coli* isolated from urban rivers and beach water. Appl Environ Microbiol **70**:4658-4665.

80. **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.

81. **Monpoeho, S., A. Dehee, B. Mignotte, L. Schwartzbrod, V. Marechal, J. C. Nicolas, S. Billaudel, and V. Ferre.** 2000. Quantification of enterovirus RNA in sludge samples using single tube real-time RT-PCR. BioTechniques **29**:88-93.

82. **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 identificaiton of fecal pollution sources in an urban watershed. J Appl Microbiol **99:**618-.

83. **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.

84. **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.

85. **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.

86. **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.

87. **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.

88. **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.

89. **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.

90. **Noblet, J. A., D. L. Young, E. Y. Zeng, and S. Ensari.** 2004. Use of fecal steroids to infer the sources of fecal indicator bacteria in the Lower Santa Ana River Watershed, California: sewage is unlikely a significant source. Environ Sci Technol **38:**6002-8.

91. **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.

92. **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.**

93. **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.

94. **Palmer, C. J., L. Xiao, A. Terashima, H. Guerra, E. Gotuzzo, G. Saldias, J. A. Bonilla, L. Zhou, A. Lindquist, and S. J. Upton.** 2003. Cryptosporidium muris, a rodent pathogen, recovered from a human in Peru. Emerg Infect Dis **9:**1174-6.

95. **Parveen, S., J. Lukasik, T. M. Scott, M. L. Tamplin, K. M. Portier, S. Sheperd, K. Braun, and S. R. Farrah.** 2006. Geographical variation in antibiotic resistance profiles of Escherichia coli isolated from swine, poultry, beef and dairy cattle farm water retention ponds in Florida. J Appl Microbiol **100:**50-7.

96. **Parveen, S., R. L. Murphree, L. Edminston, 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.

97.   **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.

98.   **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.

99.   **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.

100. **Power, M. L., J. Littlefield-Wyer, D. M. Gordon, D. A. Veal, and M. B. Slade.** 2004. Phenotypic and genotypic characterisation of encapsulated *Escherichia coli* isolated from blooms in two Austrailian lakes, Sydney, Austrailia.

101. **Puglisi, E., M. Nicelli, E. capri, M. Trevisan, and A. A. Del Re.** 2003. Cholesterol, beta-sitosterol, ergoterol, and coprostanol in agricultrual soils. J. Environ. Qual. **32:**466-471.

102. **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.

103. **Ram, J. L., R. P. Ritchie, J. Fang, F. S. Gonzalez, and Selegean.** 2004. Sequence-based source rackingof Escherichia coli based on genetic diversity of beta-glucuronidase. J Environ Qual **33:**1024-32.

104. **Resnick, I. G., and M. A. Levin.** 1981. Assessment of Bifidobacteria as indicators of human fecal pollution. Appl. Environ. Microbiol. **42:**433-438.

105. **Samadpour, M., and N. Chechowitz.** 1995. Little Soos Creek Microbial Source Tracking. Kings County Department of Public Works.

106. **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**.

107. **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.

108. **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.

109. **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**.

110. **Scott, T. M., S. Parveen, K. M. Portier, J. B. Rose, M. L. Tamplin, S. R. 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:**1089-92.

111. **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.

112. **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.

113. **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.

114. **Smith, A. W., P. L. Iversen, D. E. Skilling, D. A. Stein, K. Bok, and D. O. Matson.** 2006. Vesivirus viremia and seroprevalence in humans. J Med Virol **78:**693-701.

115. **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.

116. **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.

117. **Stewart, J. R., R. D. Ellender, J. A. Gooch, S. Jiang, S. P. Myoda, and S. B. Weisberg.** 2003. Recommendations for microbial source tracking: lessons from a methods comparison study. J. Wat. Health **01:**225-230.

118. **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.

119. **Straub, T. M., and D. P. Chandler.** 2003. Towards a unified system for detecting waterborne pathogens. J. Microbiol. Methods **53:**185-197.

120. **Straub, T. M., B. P. Dockendorff, M. D. Quinonez-Diaz, C. O. Valdez, J. I. Shutthanandan, B. J. Tarasevich, J. W. Grate, and C. J. Bruckner-Lea.** 2005. Automated methods for multiplexed pathogen detection. J Microbiol Methods **62:**303-16.

121. **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.

122. **Till, D., K. G. Field, and A. P. Dufour.** 2004. Managing risk of waterborne zoonotic disease through water quality surveillance, p. 338-348. *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.

123. **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.

124. **Wade, T. J., N. Pai, J. N. S. Eisenberg, and J. J. M Colford.** 2003. Do US EPA water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis. Environ. Health Perspect. **111:**1102-1109.

125. **Weaver, R. W., J. A. Entry, and A. K. Graves.** 205. NUmbers of fecal streptococci and Escherichia coli in fresh and dry cattle, horse, and sheep manure. Can J Microbiol **51:**847-51.

126. **Whitman, R. L., D. A. Shively, H. Pawlick, 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.

127. **Whittam, T. S.** 1989. Clonal dynamics of Escherichia coli in its natural habitat. Antonie Van Leeuwenhoek **55:**23-32.

128. **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.

129. **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.

130. **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.

131. **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.