Comparing Wastewater Chemicals, Indicator Bacteria Concentrations, and Bacterial Pathogen Genes as Fecal Pollution Indicators

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The objective of this study was to compare fecal indicator bacteria (FIB) (fecal coliforms, *Escherichia coli* [EC], and enterococci [ENT]) concentrations with a wide array of typical organic wastewater chemicals and selected bacterial genes as indicators of fecal pollution in water samples collected at or near 18 surface water drinking water intakes. Genes tested included *esp* (indicating human-pathogenic ENT) and nine genes associated with various animal sources of shiga-toxin–producing EC (STEC). Fecal pollution was indicated by genes and/or chemicals for 14 of the 18 tested samples, with little relation to FIB standards. Of 13 samples with <50 EC 100 mL^{-1}, human pharmaceuticals or chemical indicators of wastewater treatment plant effluent occurred in six, veterinary antibiotics were detected in three, and *stx*1 or *stx*2 genes (indicating varying animal sources of STEC) were detected in eight. Only the EC *eae*A gene was positively correlated with FIB concentrations. Human-source fecal pollution was indicated by the *esp* gene and the human pharmaceutical carbamazepine in one of the nine samples that met all FIB recreational water quality standards. *Escherichia coli* rfb_{O157} and *stx*2c genes, which are typically associated with cattle sources and are of potential human health significance, were detected in one sample in the absence of tested chemicals. Chemical and gene-based indicators of fecal contamination may be present even when FIB standards are met, and some may, unlike FIB, indicate potential sources. Application of multiple water quality indicators with variable environmental persistence and fate may yield greater confidence in fecal pollution assessment and may inform remediation decisions.

Recent research indicates that improved indicators of the presence, source, and potential health significance of fecal pollution are warranted (Rose and Grimes, 2001; Simpson et al., 2002; Cimenti et al., 2007; Santo Domingo et al., 2007). Quantitative fecal coliform bacteria (FC), *Escherichia coli* (EC), or enterococci (ENT) criteria are used to establish standards of microbiological quality for ambient waters designated for a variety of uses, including recreation, shellfish harvesting, drinking, and pollution control. Fecal indicator bacteria standards include the USEPA-recommended, single-sample recreational water quality criterion for ENT of 61 colony-forming units (CFU) 100 mL^{-1} and for EC of 235 CFU 100 mL^{-1} (USEPA, 2000) and the previous USEPA-recommended FC criterion of 400 CFU 100 mL^{-1} that is used in many parts of the country for National Pollutant Discharge Elimination System and Total Maximum Daily Load purposes (USEPA, 2001). The USEPA recently developed the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule) (USEPA, 2007) and set an annual mean concentration of >50 EC 100 mL^{-1} as a trigger level requiring *Cryptosporidium* monitoring for some drinking water systems using flowing stream sources.

Concentrations in water of these fecal indicator bacteria (FIB) above designated levels are presumed to indicate fecal contamination, but several recent studies indicate that various FIB may grow in the environment (Ishii et al., 2006; Walk et al., 2007) or may be associated with nonfecal sources (Whitman et al., 2003). As a further complication, recent studies have shown that FIB are poor representatives of the survival, fate, and transport of viruses and protozoa (Rose and Grimes, 2001; McQuaig et al., 2006), so human health risk may be present even when FIB concentrations meet standards. Finally, even where they do indicate fecal contamination, FIB concentrations offer no specific information on the fecal source and offer little information on potential health significance.

There have been many recent efforts to identify improved means of determining the presence and source of fecal pollution of water. These include phenotypic or genetic characterization of

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Abbreviations: CFU, colony-forming units; EC, *Escherichia coli*; ENT, enterococci; FC, fecal coliform bacteria; FIB, fecal indicator bacteria; FWA, fluorescent whitening agents; HUS, hemolytic uremic syndrome; PCR, polymerase chain reaction; STEC, shiga-toxin–producing *Escherichia coli*; WWTP, wastewater treatment plant.

FIB, detection of nonpathogenic but host-specific microbes or their genes, direct testing for host-specific pathogens or their genes, and testing for chemicals associated with specific types of fecal pollution or with human- or animal-specific waste streams (Cimenti et al., 2007; Santo Domingo et al., 2007). The direct detection of human- or animal-specific pathogens or their genes addresses source and health implications. In contrast to FIB, there are no known environmental (nongut) reservoirs for the microbial populations for which current host-specific gene assays are being developed. However, although direct analysis for pathogen genes is promising, many pathogens cannot be grown in the laboratory, and pathogens may occur seasonally or intermittently in the source. No single gene-based marker of source has been perfectly reliable, and multiple lines of evidence for source are recommended (Santo Domingo et al., 2007).

Source-specific chemical indicators may be one means of acquiring additional evidence of source (Gilpin et al., 2003; Cimenti et al., 2007). Chemical indicators of source include fecal sterols, their derivatives and ratios, and an array of chemicals (e.g., industrial and agricultural chemicals, human and veterinary pharmaceuticals, and personal care products) associated with typical waste streams (septic, manure, and wastewater treatment plant [WWTP] effluents) (Cimenti et al., 2007). Several studies have evaluated whether specific individual chemicals are dependably detected in specific waste sources or in association with specific land uses (Cimenti et al., 2007). In comparison to many microbiological methods, chemicals have the advantage of low detection limits and relatively easy analysis. However, chemicals may be degraded in the environment (Bradley et al., 2007) and may differentially persist downstream of a source (Glassmeyer et al., 2005).

Few studies have compared chemical and microbial approaches. Most studies using a combination of chemical and microbiological approaches in ambient waters have only tested FIB concentrations and have found a poor or inconsistent association between FIB concentrations and the occurrence of specific chemicals, such as caffeine, fluorescent whitening agents (FWAs), and fecal sterols (Gilpin et al., 2003; Clara et al., 2004; Isobe et al., 2002, 2004; Noblet et al., 2004; Peeler et al., 2006) or the array of chemicals detected in WWTP effluents (Glassmeyer et al., 2005). However, Young et al. (2008) investigated an urban environment highly influenced by sewage and showed that triclosan and triclocarban were positively correlated with FIB concentrations, although caffeine was not. Only two studies of ambient waters have related alternative, host-specific, microbial indicators to chemical indicators. Gilpin et al. (2003) analyzed the relation between FIB concentrations, fecal sterols, FWAs, and source-specific bacteria (*Bifidobacterium adolescentis*, *Rhodococcus coprophilus*, and a human *Bacteroides* DNA sequence), and McDonald et al. (2006) evaluated a combination of FWAs, ENT species determination, and the *esp* gene for distinguishing between human and bird fecal contamination. The perceived advantage of combined microbial and chemical methods is that their differential fate and transport improves the potential of detecting fecal pollution through a multiple-lines-of-evidence approach. In addition, unlike FIB, some chemicals and genes may indicate source. However, much more information is needed on the relations between chemical and microbial indicators of fecal pollution presence and source and on the factors that influence their detection before these newer approaches can be widely used.

The purpose of the present study was to expand on previous efforts by evaluating relations among (i) FIB concentrations, (ii) a variety of typical organic wastewater chemicals, (iii) the presence of the *esp* gene associated with human-pathogenic *Enterococcus fecalis* or *E. faecium*, and (iv) the presence of any of nine genes associated with shiga-toxin–producing *E. coli* (STEC) in ambient surface waters used as drinking water sources. The primary objective was to determine how the tested chemicals and genes compared with FIB as indicators of the presence of fecal pollution. In this study, the following variables were considered to indicate probable fecal pollution: (i) exceeding any FIB standard, (ii) the presence of any tested bacterial gene, or (iii) the presence of five detected human pharmaceuticals. The following variables were considered to indicate a potential source of pollution: the *esp* gene, STEC *stx*2 gene variants, and selected chemicals or chemical groups. Source markers were related to FIB concentrations, to each other, and to environmental variables such as watershed size and percentage of river discharge that was WWTP effluent.

Materials and Methods

Site Descriptions, Sampling Methods, and Chemical Analysis

Eighteen samples of untreated surface water were collected between July and September 2001 by the U.S. Geological Survey proximal to, or upstream of, municipal drinking water intakes in 10 US states (Table 1). The 18 sites were part of a national reconnaissance of 100 organic wastewater contaminants in raw, untreated, surface water sources of drinking water throughout the USA (Focazio et al., 2008). Each sampled site was known or suspected to have at least some human waste sources upstream of the sampling site, and for many sites the volume of effluent was quantified. The population served is an indicator of the human population in the surrounding area, and the presence of cattle or pigs in the county (or counties if the sampling point was in a river dividing two states) where the sampling point was located was determined from the National Agricultural Statistics Service as an indicator of the possibility of detecting such fecal sources. Discharge on the day of sampling was measured, and samples for water chemistry were collected on the same day as those for microbiological analysis. Chemicals analyzed, water sampling methods for chemicals, chemical analyses, and reporting levels are described in Focazio et al. (2008). Three chemical analysis methods were used, but all used compound confirmation by mass spectrometry and identification by authentic standards (Focazio et al., 2008). Compounds detected at less than the method detection limit but confirmed by the above methods were recorded as detected but were not quantified (Focazio et al., 2008).

Table 1. Sampling location

Site code	Site name	Note	Land use†	Basin size	WWTP effluent	Population served	Present in adjacent county‡	
							Cattle	Pigs
				km²	% of discharge			
GA1	Ocmulgee River, GA		MIX	5802	8.5	146,000	+	–
GA2	Oostanaula River, GA		MIX	5569	1.1	50,000	+	–
KS1	Kansas River, KS		AG	135,343	1.3	590,000	+	+
MI1	Huron River, MI		AG	1888	0.9	115,000	+	+
MI2	River Raisin, MI		AG	3017	0.1	1000	+	+
NC1	Cape Fear River, NC		MIX	8184	NA	20,000	+	+
NC2	Jordan Lake, NC	impoundment of Haw River	URB	3626	NA	170,000	+	+
NJ1	Hohokus Brook, NJ	not at intake¶	URB	42	77.4	<490,000#	–	–
NJ2	Passaic River, NJ	not at intake	URB	1974	100	<880,000	–	–
NJ3	Whippany River, NJ	not at intake	URB	181	48.5	102,000	–	–
OH1	Ohio River, OH		MIX	197,306	NA	850,000	+	+
SD1	Big Sioux River, SD		AG	7770	4.2	<150,000	+	–
SD2	Big Sioux River, SD	not at intake	MIX	10,438	5.4	<150,000	+	–
TN1	Duck River, TN		MIX	3129	0.7	39,000	+	+
TX1	Barton Springs, TX	surface pond from spring source	URB	NA§	NA	40,000	+	–
VA1	James River, VA		MIX	17,503	NA	500,000	+	–
VA2	Potomac River, VA		MIX	29,940	NA	<4,600,000	+	+
VA3	Shenandoah River, VA		MIX	2808	NA	40,000	+	–

† Predominant land use in upstream watershed. AG, agricultural land use; MIX, mixed land use; URB, urban land use.

‡ From National Agricultural Statistics Service (http://www.nass.usda.gov).

§ NA, not available.

¶ Sampling location upstream of, or otherwise removed from, drinking water intake.

Nearest county or municipality population in 2000. Population served is a proportion this number.

Bacteria Enumeration

Water samples were analyzed using standard membrane-filtration methods (APHA, 1998; USEPA, 2000) for the detection of FC (mFC medium; Difco, Detroit, MI), EC (NA-MUG medium; Difco), and ENT (mEI: mE medium [Difco] with indoxyl β-d-glucoside [Sigma, St. Louis, MO]). All media were prepared according to the manufacturers' instructions, and lot numbers of all reagents and media were recorded. Sample processing and laboratory practices followed (USEPA, 2000; APHA, 1998). For each water sample and medium, 100-, 10-, and 1-mL volumes were filtered though a 0.45-μm nylon membrane filter that was transferred to each growth medium and incubated at the appropriate temperature. Bacteria enumeration was generally based on preparations with between 20 and 80 colonies. Growth from the 100-mL mFC filter was transferred to 1 mL FC broth, and growth from the 100-mL mEI filter was transferred to 1 mL brain heart infusion broth (both Difco), with a final concentration of 10% glycerol and frozen at −70°C until analysis. These preparations, which were used for all further analyses, are hereafter referred to as the FC and ENT stocks.

Immunological Test for O157 Antigen

For every sample, 100 μL of FC stock was inoculated into Reveal for *E. coli* O157:H7 medium, and the 8-hr test was performed according to the manufacturer's instructions (Neogen, Lansing, MI). This test uses anti-O157 antibodies and detects all H serotypes of *E. coli* O157 (Power et al., 2000); therefore, all positive results are referred to as detection of *E. coli* O157. The procedural limits of this analysis were evaluated by preparing samples with a known concentration of *E. coli* O157:H7 (ATCC 700728, nontoxigenic) cells as the sole bacterial type in the sample and against a typical FC culture background. We detected *E. coli* O157:H7 in samples inoculated with ≥100 cells with no FC background but found that FC interfere with the test when the ratio of FC cells to *E. coli* O157:H7 ATCC 700728 cells is ≥10,000:1.

DNA Extraction

DNA was extracted from a volume of FC stock equivalent to 5.0×10^8 cells (1–250 μL). Cells were collected by centrifugation, and DNA was extracted using a 3-hr proteinase K/SDS lysis time with the Qiagen DNAeasy DNA extraction kit (Qiagen, Valencia, CA). Fecal coliform bacteria DNA was eluted into two 200-μL volumes of elution buffer and stored at –20°C until analysis. DNA was extracted from 100 μL of ENT stocks. Cells were collected by centrifugation, rinsed once with Tris-EDTA buffer (pH 8.0), resuspended, and subjected to alternate freezing and boiling for three 10-min cycles. Samples were centrifuged to remove cellular debris, and 150 μL of supernatant was transferred to a new tube for analysis.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) was conducted using a PerkinElmer GeneAmp PCR System 2400 Thermal Cycler (PerkinElmer, Boston, MA). All reaction mixtures were 25 μL final volume. Polymerase chain reaction fragments were separated on 2% agarose gels in Tris Acetate EDTA buffer and stained for 15 min in 0.5 μg mL^{-1} ethidium bromide solution. Fragments were visualized using a Foto/prep transilluminator (Fotodyne, Hartland,WI), imaged using a Kodak EDAS 290 Zoom digital camera, and analyzed using the Kodak 1D-gel image analysis software. Genes, detection limits, and control organisms are described in Table 2. Primers used and reaction conditions are described in Table 3.

Table 2. Genes tested, gene functions, detection limit by polymerase chain reaction, and polymerase chain reaction control.

Gene	Encodes for	Detection limit (cells)	Positive control
E. coli 16S	*E. coli* 16S rDNA, a conserved gene in all *E. coli* used as an internal control	112	*E. coli* O157:H7 ATCC† 35150
*eae*A	intimin protein for attachment of *E. coli* to intestinal cells	112	*E. coli* O157:H7 ATCC 35150
*stx*1	shiga-toxin 1 protein	112	*E. coli* O157:H7 ATCC 35150
*stx*2	shiga-toxin 2 protein variant	112	*E. coli* O157:H7 ATCC 35150
rfb_{O157}	O157 surface protein in *E. coli* with the O157 serotype		*E. coli* O157:H7 ATCC 35150 or *E. coli* ATCC 700728
$fliC_{H7}$	H7 flagella protein in *E. coli* with the H7 serotype		*E. coli* O157:H7 ATCC 35150 or *E. coli* ATCC 700728
*stx*2c	shiga-toxin 2 protein variant	20–200	*E. coli* O91:H21 B2F1‡
*stx*2d	shiga-toxin 2 protein variant	20–200	*E. coli* O118:H12 EH250‡
*stx*2e	shiga-toxin 2 protein variant	20–200	*E. coli* O138 E145‡
*stx*2f	shiga-toxin 2 protein variant	2–20	*E. coli* O128 HI8‡
esp	*Enterococcus* surface protein, associated with human disease	34	*Ent. faecalis* E-53

† ATCC, American Type Culture Collection.

‡ STEC Center, Michigan State Univ.

Multiplex PCR was used to detect STEC. This analysis was designed to detect four genes: *eae*A, *stx*1, *stx*2, and the 16S rDNA of EC. The latter gene was used as an internal positive control. The *stx*2 primers used in this assay encompass most shiga-toxin variants, with the exception of *stx*2d (EH250) and the rare *stx*2 g (Ziebell et al., 2002). If *stx*2 was detected by this multiplex reaction and if further assays did not reveal one of the *stx*2c-*stx*2f variants, the *stx*2 variant was assumed to be present. Reaction mixtures contained 3 to 160 ng of template DNA. A separate multiplex PCR assay was designed to detect the *rfb* gene for the *E. coli* O157 serotype (rfb_{O157}) and $fliC_{H7}$ gene for the H7 serotype (Tables 2 and 3). Reaction mixtures contained 10 to 100 ng of template DNA. Individual PCR reactions were used to detect shiga-toxin 2 variants *stx*2c, *stx*2d, *stx*2e, and *stx*2f and the ENT gene *esp* (Tables 2 and 3). Reaction mixtures contained 25 to 165 ng of template DNA per reaction. All reagents were from Promega (Madison, WI) (rfb_{O157} and $fliC_{H7}$) or Applied Biosystems (Foster City, CA) (all other reactions).

Quality Assurance/Quality Control

Several steps were taken to assure the quality of each PCR reaction, following recommendations provided by USEPA (2004). For approximately every 20 samples of any given PCR reaction, PCR-positive controls (extra reaction with 10 ng of DNA from the positive control strain) and PCR-negative controls (blank water replaced template DNA) were included. Environmental matrix issues were diminished because we analyzed the FC or ENT culture from the original water sample; nevertheless, a matrix spike (addition of positive DNA to a previously analyzed negative sample) was performed approximately every 40 reactions for the *stx*2 variant and *esp* gene assays. Polymerase chain reaction inhibition controls (16S rDNA of *E. coli*) were run with every reaction of the STEC multiplex PCR.

Detection limits for the STEC and *esp* gene PCR assays were calculated as in USEPA (2004). Polymerase chain reaction assay detection limits were determined empirically by establishing a relationship between cell number and DNA concentration. Using a serial dilution series on DNA of a known concentration, DNA was diluted to levels that corresponded to values between 1 and 10,000 cells, and PCR was performed using the standard protocol. The detection limit was determined to be the last detectable dilution (and corresponding cell number) using the standard method. Using both procedures, detection limits agreed within a range of ±30 cells. Detection limits for PCR assays are indicated in Table 2. Because the PCR assays were performed on enrichment cultures, the exact number of genes or organisms in the original water sample cannot be determined. However, any positive result by PCR reflects that at least one organism with the indicated gene was present in 100 mL or less of the original water sample.

Results and Discussion

Indicators of the Presence of Fecal Contamination

Of 100 chemicals tested, 41 were detected in at least one sample (Table 4), and the remaining 59 (Table 5) were not detected. We interpreted carbamazepine, codeine, dehydronifedipine, diltiazem, or fluoxetine to indicate a uniquely fecal source because these human pharmaceuticals are consumed and have no external uses and have been detected in 73 to 91% of WWTP effluents at concentrations significantly greater than upstream locations (Glassmeyer et al., 2005). In contrast, the human pharmaceutical metabolites (1,7-dimethylxanthine derived from metabolism of caffeine; cotinine derived from nicotine) did not occur in WWTP effluent at concentrations significantly greater than upstream sampling locations (Glassmeyer et al., 2005). Therefore, for the purposes of this study, we assumed the five human pharmaceuticals indicated probable fecal contamination but that the metabolites did not.

There was an inconsistent relation between FIB concentrations and chemical- or gene-based indicators of fecal pollution (Table 4). Three samples (NJ1, TX1, and MI2) exceeded all FIB recreational water quality standards. Samples NJ1 and MI2 contained multiple pharmaceuticals or metabolites and gene-based indications of fecal pollution, but for TX1, no pharmaceuticals and only the *eae*A gene were detected. In contrast, of nine samples (NJ3, NC2, GA1, OH1, VA1, VA2, TN1, KS1, and MI1) that met FIB recreational water quality standards, gene-based indications of fecal pollution were detected in five (NJ3, OH1, VA1, VA2, and MI1), and pharmaceuticals were detected in one (NJ3). For the 13 samples with <50 EC 100 mL^{-1}, only four (NC2, GA1, TN1, and KS1) had no detection of any gene or any of the five pharmaceuticals. However, enrofloxacin, a veterinary antibiotic for which a fecal

Table 3. Polymerase chain reaction primer sequences and reaction conditions.

Gene	Primers 5′–3′	Primer concentration	PCR† buffer	$MgCl_2$ concentration	Initial denaturing		Denaturing		Annealing		Extension		Final Extension		Cycles	Reference
					Temp.	Time	Temp.	Time	Temp.	Time	Temp.	Time	Temp.	Time		
		μmol L^{-1}		mmol L^{-1}	°C	min	°C	min	°C	min	°C	min	°C	min		
STEC‡ multiplex PCR																
*stx*1	F: ACA CTG GAT GAT CTC AGT GG	0.05	Buffer II	3	95	10:00	95	0:30	56	0:40	72	1:30	72	5:00	35	Fagan et al, 1999; Gannon et al.,1992; Sabat et al., 2000
	R: CTG AAT CCC CCT CCA TTA TG	0.05														
*stx*2	F: CCA TGA CAA CGG ACA GCA GTT	0.05														
	R: CCT GTC AAC TGA GCA GCA CTT TG	0.05														
*eae*A	F: GTG GCG AAT ACT GGC GAG ACT	0.05														
	R: CCC CAT TCT TTT TCA CCG TCG	0.05														
E. coli 16s rDNA	F: GGA AGA AGC TTG CTT CTT TGC TGA C	0.025														
	R: AGC CCG GGG ATT TCA CAT CTG ACT TA	0.025														
E. coli O157 multiplex PCR																
rfb_{O157}	F: CGT GAT GAT GTT GAG TTG	0.1	Buffer II	5	95	10:00	94	1:00	53	1:00	72	1:00	72	5:00	30	Maurer et al., 1999; Osek, 2003
	R: AGA TTG GGT TGG CAT TAC TG	0.1														
$fliC_{H7}$	F:GCTGCAACGGTAAGTGAT	0.05														
	R:GGCAGCAAGCGGGTTGGT	0.05														
*E. coli stx*2 variant PCR																
stx2c	F:GCG GTT TTA TTT GCA TTA GT	0.5	Gold	1.5	95	10:00	94	0:30	58	0:30	72	0:30	72	5:00	40	Osek, 2003
	R:AGT ACT CTT TTC CGG CCA CT	0.5														
stx2d	F:GGT AAA ATT GAG TTC TCT AAG TAT	0.5	Gold	1.5	95	10:00	94	0:30	58	0:30	72	0:15	72	5:00	35	Osek, 2003
	R:CAG CAA ATC CTG AAC CTG ACG	0.5														
stx2e	F:ATG AAG AAG ATG TTT ATA GCG	0.5	Gold	5.0	95	10:00	94	0:30	59	0:30	72	0:30	72	5:00	35	Osek, 2003
	R:TCA GTT AAA CTT CAC CTG GGC	0.5														
stx2f	F:AGA TTG GGC GTC ATT CAC TGG TTG	0.5	Buffer II	1.5	95	10:00	94	0:30	57	1:00	72	1:00	72	5:00	40	Osek, 2003
	R:TAC TTT AAT GGC CGC CCT GTC TCC	0.5														
Enterococcus pathogen PCR																
esp	F:TTG CTA ATG CTA GTC CAC GAC C	0.5	Buffer II	2.5	95	10:00	94	0:15	66	1:00	-	-	72	5:00	45	Shankar et al., 1999
	R:GCG TCA ACA CTT GCA TTG CCG AA	0.5														

† PCR, polymerase chain reaction.
‡ STEC, shiga-toxin–producing *Escherichia coli*.

Table 4. Chemical and microbiological indicators of the presence and/or source of fecal pollution at the sampled sites.

	Site																	
	URB†					MIX									AG			
	NJ2	NJ1	NJ3	TX1	NC2	GA1	SD2	GA2	NC1	OH1	VA1	VA2	VA3	TN1	SD1	KS1	MI1	MI2
Bacteria concentrations																		
E. coli/100 mL	3	**310‡**	40	**627**	0	0	23	228	20	13	22	15	36	3	50	25	16	**690**
Enterococci/100 mL	**101**	**128**	60	**3000**	1	0	**84**	**110**	**105**	9	40	26	**138**	25	**81**	56	38	**1109**
Fecal coliforms/100 mL	**510**	**620**	190	**2700**	0	1	40	250	24	13	290	29	145	4	90	25	16	**1000**
Bacterial genes§																		
*eae*A (human and animal)	D¶	D	D	D	–	–	D	D	D	–	D	D	D	–	D	–	–	D
esp (human)	D	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
*stx*2f (pigeons)	D	D	D	–	–	–	–	–	D	–	–	–	–	–	–	–	D	D
*stx*2 (cattle)	–	–	–	–	–	–	–	–	–	–	–	–	D	–	D	–	–	–
*stx*2c (cattle)	–	–	–	–	–	–	–	D	–	–	–	–	–	–	–	–	–	–
*stx*1 (cattle)	–	–	–	–	–	–	D	–	–	–	–	–	–	–	–	–	–	D
*rfb*O157 (cattle)	–	–	–	–	–	–	–	D	–	–	–	–	–	–	–	–	–	–
*stx*2e (pigs)	–	–	–	–	–	–	–	–	–	D	–	D	–	–	–	–	D	–
Human pharmaceuticals																		
Carbamazepine (0.011)#	D	D	D	–	–	–	–	–	–	–	–	–	D	–	–	–	–	+
Codeine (0.24)	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dehydronifedipine (0.01)	–	D	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Diltiazem (0.012)	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fluoxetine (0.018)	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Human pharmaceutical metabolites																		
1,7-dimethylxanthine (0.018)	–	D	–	–	–	–	–	–	–	D	–	–	+	–	–	–	+	D
Cotinine (0.023)	+	+	+	–	+	–	–	–	+	+	+	–	+	+	+	+	+	+
Wastewater chemicals††																		
AHTN, tonalide (0.5)	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Benzophenone (0.5)	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ethyl citrate (0.5)	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HHCB, galaxolide (0.5)	+	D	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
tri(2-chloroethyl) phosphate (0.5)	+	+	+	–	+	–	–	–	+	–	–	–	–	–	–	–	+	+
tri(dichlorisopropyl) phosphate (0.5)	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
tributyl phosphate (0.5)	–	+	–	–	D	–	–	–	D	–	–	–	–	–	–	–	–	–
triclosan (1)	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Human antibiotics																		
Erythromycin H_2O (0.02)	–	D	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ciprofloxacin (0.02)	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Trimethoprim (0.014)	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Veterinary antibiotics																		
Enrofloxacin (0.01)	–	–	–	–	–	D	–	–	–	–	–	–	–	D	–	–	–	–
Sarafloxacin (0.02)	–	–	–	–	–	–	–	–	–	–	D	–	–	–	–	–	–	–
Sterols																		
Beta-sitosterol (2)	–	+	–	–	+	–	+	–	+	–	–	+	–	–	+	–	+	–
Cholesterol (2)	–	+	+	–	+	–	+	–	+	+	–	+	–	+	+	+	+	–
Coprostanol (2)	–	+	+	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–
Pesticides/herbicides																		
Bromacil (0.5)	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chlorpyrifos (0.5)	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Diazinon (0.5)	+	+	+	D	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Metalaxyl (0.5)	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Metolachlor (0.5)	–	–	+	–	+	–	+	–	+	+	–	+	+	+	–	+	–	+
Prometon (0.5)	+	–	+	–	–	+	–	–	+	–	–	+	+	+	–	+	–	–
Other chemicals																		
4-tert-octylphenol (1)	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
5-methyl-1H-benzotriazole (2)	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Caffeine (0.104)‡‡	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Diethoxynonylphenol-total (5)	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–
Diethoxyoctylphenol-total (1)	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ethanol 2-butoxy-phosphate (0.5)	+	D	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–
Indole (0.5)	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Methyl salicylate (0.5)	–	+	+	–	–	–	–	–	–	–	+	+	–	–	+	–	–	–
N,N,-diethyltoluamide (0.5)	–	+	–	–	+	–	–	–	+	–	–	–	–	–	–	–	+	–
Para-cresol (1)	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Skatol (1)	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tetrachloroethylene (0.5)	–	–	–	–	–	–	D	–	–	–	–	–	–	–	–	–	–	–

† Sites are arranged according to land use. URB, urban land use; MIX, mixed land use; AG, agricultural land use.

‡ Bold values exceed recreational water quality standards.

§ The *stx*2d gene was never detected and the $fliC_{H7}$ gene was detected in every sample.

¶ D, detected (for chemicals: present at concentration > RL, reporting level); +, detected but not quantified; –, not detected.

Value in parentheses is RL in μg L^{-1}.

†† Chemicals detected in 100% of WWTP effluents in Glassmeyer et al. (2005).

‡‡ RL censored to adjust for blank contamination (Focazio et al., 2008).

Table 5. Chemicals analyzed but not detected.

Chemical	RL†	Chemical	RL
	μg L^{-1}		μg L^{-1}
Human and veterinary antibiotics		Other chemicals	
Azithromycin	0.023	1,4-dichlorobenzene	0.5
Carbodox	0.1	1-*methyl* naphthalene	0.5
Chlortetracycline	0.05	2,6-*dimethyl* naphthalene	0.5
Demeclocycline	0.05	2-*methyl* naphthalene	0.5
Doxycycline	0.1	3-*tert*-butyl-4-hydroxyanisole	5.0
Lincomycin	0.05	4-cumylphenol	1.0
Methotrexate	0.05	4-*n*-octylphenol	1.0
Minocycline	0.05	4-octylphenol monoethoxylate	1.0
Norfloxacin	0.02	Acetophenone	2.0
Oxytetracycline	0.1	Anthracene	0.5
Roxithromycin	0.03	Anthraquinone	0.5
Sulfadimethoxine	0.05	Benzo(a)pyrene	0.5
Sulfamerazine	0.05	Bisphenol A	1.0
Sulfamethazine	0.05	Camphor	0.5
Sulfamethizole	0.05	d-limonene	0.5
Sulfamethoxazole	0.023	Fluoranthene	0.5
Sulfathiazole	0.1	Isoborneol	0.5
Tetracycline	0.05	Isophorone	0.5
Tylosin	0.05	Isoproplylbenzene	0.5
Virginiamycin	0.1	Isoquinoline	0.5
		Menthol	0.5
Human pharmaceuticals		Naphthalene	0.5
Albuterol	0.029	*Para*-nonylphenol	5.0
Cimetidine	0.007	Pentachlorophenol	2.0
Diphenhydramine	0.01	Phenanthrene	0.5
Gemfibrozil	0.015	Phenol	2.0
Ranitidine	0.01	Pyrene	0.5
Warfarin	0.001	Triphenyl phosphate	0.5
Acetaminophen	0.009		
Ibuprofen	0.018		
Sterols			
Stigmastanol	2.0		
Pesticides/herbicides			
Carbaryl	1.0		
Dichlorvos	1.0		

† RL, reporting level.

source is possible (Sarmah et al., 2006), was detected in GA1 and TN1.

The *eae*A gene was the only gene-based indicator of fecal pollution that was significantly positively correlated (Pearson product moment correlation, 16 df) with the (log10) concentrations of any FIB (ENT 0.52, $p < 0.05$; FC 0.70, $p < 0.01$). Nevertheless, the *eae*A gene was detected in three samples (NJ3, VA1, and VA2) that met all FIB water quality standards, including the <50 EC 100 mL^{-1} LT2 Rule trigger level. The *eae*A gene produces a protein (intimin) that is needed for attachment to gut cells. This gene can be detected in EC from many different animals and from humans (Ahmed et al., 2007) and was detected more frequently than other EC virulence genes in water samples in (Ahmed et al., 2007). The positive correlation with FIB concentrations may reflect multiple sources and an increased probability of having one or more true fecal sources as the numbers of FIB increase. The five pharmaceuticals occurred too infrequently to establish meaningful associations with FIB concentrations. Carbamazepine occurred in four samples that exceeded one or more FIB standards and in one sample that did not exceed FIB standards.

In our study, which examined ambient waters in various land-use environments with a wide range of FIB concentrations, fecal pollution was indicated by gene-based and/or chemical-based markers for 14 of the 18 tested samples, with little relation to FIB standards. These findings are consistent with previous studies in which FIB concentrations have been poorly or inconsistently related to chemical-based (Gilpin et al., 2003; Isobe et al., 2002; Noblet et al., 2004; Glassmeyer et al., 2005; Peeler et al., 2006) or gene-based (McQuaig et al., 2006) indicators of fecal pollution. As elaborated below, factors such as variable occurrence of a chemical or gene within the source, variable persistence in the environment, and environmental conditions at the time of sampling may influence relations among FIB concentrations and the occurrence of chemicals or bacterial genes, resulting in inconsistencies among indicators. Nevertheless, the current study adds to the growing body of evidence that fecal pollution may be present even when FIB concentrations are lower than recommended standards and that alternate indicators may enhance the ability to detect or confirm the presence of fecal pollution.

Indicators of the Source of Contamination

Bacterial Genes as Indicators of Source

Unlike FIB, some genes may indicate a potential source of fecal pollution. Of the 18 water samples, two had no EC, one had no ENT to test, and two more had no bacterial genes other than the16S rDNA and *fli*C_{H7} genes of EC detected, even though EC and ENT were present. The *fli*C_{H7} gene indicates the presence of the H7 serotype of EC, and its detection in every sample likely reflects the occurrence of many different O serotypes with this H antigen. Of the remainder, bacterial genes indicated potential human (*esp*) sources in two samples from urban watersheds, pigeon wastes in six samples from all land-use categories, and pig or cattle sources in eight samples from mixed or agricultural land-use watersheds where pigs or cattle were present.

The *esp* gene, which indicates human pathogenic ENT, was detected in two samples (NJ2 and NJ3) with urban land use, a relatively large human population in a small watershed, and consequently high percentages of effluent in discharge. *Enterococcus faecium* and *Ent. fecalis* that carry the enterococcal surface protein (*esp*) gene are human pathogens (Shankar et al., 1999). The *esp* gene was significantly correlated with two other microbial markers of human source (human *Bacteroides* DNA sequences and human polyomavirus) in environmental waters (McQuaig et al., 2006) and has been detected in waters with human enteroviruses (Jenkins et al., 2005). Our results agree with the interpretation that the *esp* gene is an indicator of human inputs because both samples had a high percentage of WWTP effluent, were from urban land-use settings, and contained human-use pharmaceuticals or antibiotics and wastewater chemicals. These results demonstrate the utility of this gene as a bacterial source-tracking tool and help to confirm the utility of selected wastewater chemicals as indicators of fecal pollution source. However, the *esp* gene was not detected in every sample with a human/wastewater chemical signature. The primer set that we used was recently found to detect the *esp* gene in 100% of raw sewage samples, in 30% of septic systems, and in 80% of

active pit toilets but also in 7.7% of tested animal feces from dogs, gulls, mice, and songbirds but not from deer, cats, geese, or raccoons (Whitman et al., 2007). The results from Whitman et al. (2007) indicate the transient or intermittent occurrence of pathogens in human fecal wastes and the potential for the occurrence of "human-specific" bacterial genes in animals, especially animals with the potential for close associations with humans. Our findings are consistent with the observation that no source marker gene has been found to be a perfect indicator of source (Santo Domingo et al., 2007) and demonstrate that combined information, such as watershed characteristics and the occurrence of specific genes and chemicals, may yield greater confidence in source interpretation.

Shiga-toxin genes indicative of STEC were detected in 12 samples. Shiga-toxin–producing EC are a major cause of gastrointestinal disease in humans, and some strains may lead to severe disease, such as hemolytic uremic syndrome (HUS) or hemorrhagic colitis (Nataro and Kaper, 1998). These bacteria are carried by various animals, and specific shiga-toxin gene variants have been associated with specific animals and human health outcomes (Boerlin et al., 1999; Beutin et al., 1993, 2004; Friedrich et al., 2002). With the exception of the *stx*2f gene, animal-source genes were detected only in mixed or agricultural land-use watersheds. The *stx*2f gene, first detected in pigeons (Schmidt et al., 2000), is rarely detected in STEC from humans with gastrointestinal disease (Friedrich et al., 2002). The occurrence of the *stx*2f gene was significantly positively correlated with the percentage of discharge that was effluent (0.60; $p < 0.01$), and every sample positive for the *stx*2f gene contained one or more wastewater chemicals or human pharmaceuticals. The correlation of the *stx*2f gene with effluent does not necessarily mean that effluent is the source but may reflect an urban setting where pigeon feces could readily pollute surface/storm water runoff.

The *stx*1 gene has been statistically associated with cattle isolates (Boerlin et al., 1999). The *stx*2e gene is associated with STEC isolates from pigs with pig edema disease (Fratamico et al., 2004), but this disease may not be present in all host populations. Neither gene is common in STEC isolates from humans with gastrointestinal disease (Friedrich et al., 2002; Beutin et al., 2004). The five *stx*1 and *stx*2e detections (SD2, OH1, VA2, MI1, and MI2) were at sites with mixed or agricultural land use and where the appropriate source animals were present in the adjacent county. However, these gene markers were not detected at every site with cattle or pigs present, and human pharmaceuticals or wastewater chemicals were detected at MI1 and MI2. The *stx*2e gene was detected only at sites with low concentrations of FIB that met all water quality standards.

The *stx*2 and *stx*2c variants are the most common variants detected in STEC isolates from humans with gastrointestinal disease or HUS (Friedrich et al., 2002; Beutin et al., 2004; De Baets et al., 2004), and the typical reservoir for such STEC is cattle (Brett et al., 2003). The *stx*2 or *stx*2c gene variants were detected, along with the *eae*A gene, in three samples (GA2, VA3, and SD1) from mixed or agricultural land-use watersheds and for which cattle were present in the adjacent county. The *stx*2 or *stx*2c variants typically occur with the *eae*A gene in the *E. coli* O157:H7 serotype. The *stx*2c gene was detected in the GA2 sample along with immunological (Reveal for *E. coli* O157:H7; not shown) and PCR (rfb_{O157}) evidence of the presence of the O157 serotype of *E. coli*. Although our assay cannot indicate if these genes were all present in the same organism, the GA2 sample seems to be a reservoir for STEC genes and serotypes associated with a cattle source. The GA2 sample was noteworthy for the absence of any chemical detections; however, few chemicals that might be associated with agricultural sources were analyzed.

Chemicals as Indicators of Source

For the purposes of this study, detected chemicals were grouped according to the source categories shown in Table 4. Few animal-specific or agricultural-source chemicals were analyzed. Interpretation of source for many of the chemicals analyzed requires consideration of multiple environmental pathways. Most of the chemicals analyzed (with the exception of the pharmaceuticals) could feasibly come from surface runoff, "gray water" effluents, or agricultural or industrial wastes that are not uniquely linked to a fecal source. The vast majority of the chemicals in Table 4 have been detected in WWTP effluents (Glassmeyer et al., 2005), biosolids destined for land application (Kinney et al., 2006), or septic system wastes (Conn et al., 2006). Therefore, with some exceptions, most of the chemicals detected may represent some source that could contain fecal waste. The wastewater chemicals detected were found in 100% of WWTP effluents tested by Glassmeyer et al. (2005) and at concentrations significantly greater than those found at upstream sampling points. The human pharmaceuticals were previously addressed as indicators of fecal pollution and are likely unequivocal indicators of human-source contributions to surface waters. However, some human-use antibiotics may be used in companion animals, horses, or some livestock based on veterinary discretion. Veterinary antibiotics have no human uses but may occur in urban wastewater streams due to use in companion animals. Many human or veterinary antibiotics may be applied topically, so their occurrence does not necessarily indicate a fecal pathway. Among the sterols, β-sitosterol is not specific to a given animal source but has been associated with rural land uses (Isobe et al., 2002) and with bird fecal pollution (Noblet et al., 2004). Among the compounds labeled "other chemicals" in Table 4, several were detected infrequently in WWTP effluents, and only 5-methyl-1H-benzotriazole, diethoxynonylphenol, and N,N,-diethyltoluamide were significantly elevated in effluents in comparison to upstream samples (Glassmeyer et al., 2005). Caffeine has been found to occur naturally in some waters (Peeler et al., 2006), and Young et al. (2008) found it less correlated with sewage-derived bacteria than triclosan and triclocarban.

Of 13 samples with <50 EC 100 mL^{-1}, six had indications of probable human-source pollution (human pharmaceuticals, wastewater chemicals, or human-use antibiotics). Probable human-source chemicals were detected in three of nine samples that met all FIB recreational water quality standards. This analysis did not consider the human pharmaceutical metabolites because these were detected in other research upstream of WWTP effluents (Glassmeyer et al., 2005). If 1,7-dimethylxanthine and cotinine were considered, then 10 of the 13 samples with <50 EC 100 mL^{-1} would have evidence of human pollution sources.

Nonhuman sources of pollution are more difficult to determine with this data set because few chemicals were analyzed that could be unequivocally assigned to other potential sources. Three of the samples with <50 EC 100 mL^{-1} had detections of veterinary antibiotics. These three samples had no compelling human-source chemical signature. Chemical detections, in combination with bacterial genes and land use, indicated multiple potential sources for many sites. For example, the corn and soybean herbicide metolachlor, which has few urban uses, was detected in three samples along with carbamazepine, for which an agricultural source is unlikely. This is reasonable to expect in ambient waters, where multiple factors (discussed below) affect the probability of detecting a given chemical and where there may exist a multitude of sources for any given watershed sampling point.

Indications of Potential Risk

Unlike FIB, some genes and chemicals may suggest specific health risks. Each sample in our study was collected proximal to or upstream of a drinking water intake. Although we evaluated the *esp* and *stx*2 variant genes as indicators of source, these genes are also associated directly and indirectly with human pathogens. The *esp* gene occurs in human pathogenic ENT, and the presence of this gene in water indicates some potential for contact-associated infection directly due to these organisms. In a recent study, some water samples in which the *esp* gene was detected also harbored human enteroviruses (Jenkins et al., 2005). In our study, the *esp* gene occurred in two source-water samples along with chemicals indicating human-source pollution. These samples could be construed as potential reservoirs for other human-source pathogens, such as viruses. Likewise, recent studies have indicated that HUS and severe diarrhea are significantly associated with STEC (particularly *E. coli* O157:H7) carrying the *stx*2 and *stx*2c variants along with the *eae*A gene. *Escherichia coli* O157:H7 has a low infectious dose, possibly less than 100 cells (Tuttle et al., 1999). Our assay would indicate the possibility of at least one *E. coli* O157:H7 organism in 100 mL of GA2 source water, and because our assay was performed on the FC stock culture from the original 100 mL water sample, the organism(s) carrying the rfb_{O157}, *stx*2c, and *eae*A genes in this sample were viable in the sampled water. In contrast to the samples with *esp* detections, the GA2 sample could be construed as a reservoir for zoonotic pathogens, including pathogens associated with cattle, such as *Cryptosporidium*. Although enterococci and *E. coli* are likely removed in the treatment process, our results indicate a potential for human health risk if treatment failure occurs or if treatment fails to remove potential pathogens such as viruses and *Cryptosporidium*. No chemicals were detected in the GA2 sample. This finding points out the need for additional, nonsewage-, nonwastewater-related chemical indicators.

Factors Influencing Indicator Detection

The set of surface water samples analyzed was selected to determine surface water quality at drinking water intakes. The sampling points included a wide range of basin size, hydrology, and geography. The samples were not replicated over time, and several variables likely influenced our results. Among the factors known to influence chemical and microbiological indicator detection in water at any sampling time are (i) the relative occurrence of indicator within source, (ii) environmental degradation or dilution of indicator, (iii) proximity and intensity of source, and (iv) hydrologic variables. Each of these factors may have influenced detections in this study and may have contributed to inconsistencies among indicators.

Not all of the chemicals tested are present in WWTP effluents (Glassmeyer et al., 2005). Chemical presence depends on use within the human population contributing to the effluent. In addition, some of the tested chemicals are more persistent than others downstream of the effluent (Glassmeyer et al., 2005), and some chemicals may be degraded in the environment (Bradley et al., 2007). *Escherichia coli* are a primary target of WWTP processes. In our study, the ratio of EC/FC was negatively correlated with % effluent (−0.82; $p < 0.01$), but the FC/ENT ratio was significantly positively correlated with % effluent (0.94; $p < 0.01$). Our sample set was small and not replicated. However, if such patterns were borne out in further studies, the relative occurrence of FC, EC, and ENT in WWTP effluents or other sources might influence the probability of detecting specific genes, such as *esp*, associated with one of these FIB groups. Likewise, the occurrence of the *stx*2e gene depends on the relative occurrence of the swine-pathogenic *E. coli* that carry this gene. In addition, signature genes for various sources are likely to show temporal or geographic variation in occurrence. For example, STEC are most common in young animals and in the summer months in the Northern Hemisphere (Bach et al., 2002). Differences in occurrence within the source may be one reason why the *esp* gene was not detected in every sample with a human chemical signature and why cattle- or pig-specific genes were not detected in every sample for which these animals were present in surrounding counties.

Proximity and intensity of a given source to the sampling point may influence chemical or microbiological detections. In our study, proximity of the WWTP effluent to the sampling point was not determined. However, the percentage of discharge that was effluent, a measure of intensity, was determined for several sites. The percentage of discharge that was effluent was negatively correlated with basin size in this sample set (−0.61; $p < 0.01$), primarily influenced by the high percentages for the three relatively small, highly populated NJ basins. Consistently, in our study, the greatest concentration and number of chemicals detected was in one of the smallest basins (NJ1) with a high percentage of river discharge that was WWTP effluent. Glassmeyer et al. (2005) observed that AHTN (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene) and HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8-hexamethylcyclopenta-gamma-2-benzopyran) were not persistent downstream of WWTP effluents, but carbamazepine was. The NJ1 sample was the only sample in which all three chemicals were detected and in which HHCB was detected at a concentration of >0.5 μg L^{-1}. This pattern indicates that not only was WWTP effluent a relatively large percentage of river discharge at NJ1, but also that the effluent outfall was likely more proximal to the sampling point than for other sites. Unfortunately, information on relative volumes and proximity to a sampling point are typically unknown for septic, biosolids, or animal sources of pollu-

tion. One intense proximal source, such as overland flow of animal wastes through a small creek or a leaking septic system adjacent to the sampling point, may overwhelm signatures from sources further upstream. The relative influence of proximal as opposed to more distant location for other sources could not generally be determined for our study.

Timing of sampling with respect to streamflow variation also influences the detection of many of the chemicals we studied (Kolpin et al., 2004). In this study, samples were collected between July and September, when streamflow is often naturally at annual lows and when WWTP effluents, if present, likely comprise a larger portion of discharge than when streamflow is naturally high. Timing of sampling with respect to storm events would also influence the likelihood of detecting constituents such as agricultural chemicals or manure-derived bacteria delivered by rainfall runoff.

Conclusions

This study was the first to compare FIB concentrations with a wide array of typical organic wastewater chemicals and selected bacterial genes as indicators of fecal pollution in water samples collected at or near surface water drinking water intakes. Fecal pollution was indicated by bacterial genes and/or chemicals in several water samples that met all FIB standards. As reported by others, although for fewer constituents, the occurrence of genes and chemicals indicating fecal pollution was generally unrelated to FIB concentrations. Reliance on a single type of fecal-pollution indicator (e.g., FIB concentrations or chemicals) may not provide a complete assessment of the presence of fecal pollution in surface water because each indicator varies in occurrence, persistence, and patterns of environmental transport. Application of multiple water quality indicators with variable environmental persistence and fate may yield greater confidence in source-water quality assessment. Furthermore, unlike FIB, some chemicals and bacterial genes may be useful as indicators of specific sources of fecal pollution, thereby informing remediation measures. This study contributes to the growing body of literature on potential alternative indicators of the presence of fecal pollution and identifies some of the inconsistencies and potential variables that may influence their utility. Future studies in which watersheds are selected for their potential fecal pollution characteristics and samples are designed to identify occurrence and transport characteristics of multiple indicators over time and under varying hydrologic conditions would be beneficial to clarify the relative merits of chemical- and gene-based approaches. In addition, more chemical indicators associated with animal wastes are needed to strengthen the weight-of-evidence approach for fecal pollution detection. Eventually, specific chemical- and gene-based indicators of fecal pollution may be identified that are practical, reliable, and cost effective for fecal pollution assessment.

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