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Geographic Setting Influences Great Lakes Beach Microbiological Water Quality

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Supporting Information

ABSTRACT: Understanding of factors that influence *Escherichia coli* (EC) and enterococci (ENT) concentrations, pathogen occurrence, and microbial sources at Great Lakes beaches comes largely from individual beach studies. Using 12 representative beaches, we tested enrichment cultures from 273 beach water and 22 tributary samples for EC, ENT, and genes indicating the bacterial pathogens Shiga-toxin producing *E. coli* (STEC), *Shigella spp.*, *Salmonella spp*, *Campylobacter jejuni/coli*, and methicillin-resistant *Staphylococcus aureus*, and 108−145 samples for *Bacteroides* human, ruminant, and gull source-marker genes. EC/ENT temporal patterns, general *Bacteroides* concentration, and pathogen types and occurrence were regionally consistent (up to 40 km), but beach catchment variables (drains/creeks, impervious surface, urban land cover) influenced exceedances of EC/ENT standards and detections of *Salmonella* and STEC. Pathogen detections were more numerous when the EC/ENT Beach Action Value (but not when the Geometric Mean and Statistical Threshold Value) was exceeded. EC, ENT, and pathogens were not necessarily influenced by the same variables. Multiple *Bacteroides* sources, varying by date, occurred at every beach. Study of multiple beaches in different geographic settings provided new insights on the contrasting influences of regional and local variables, and a broader-scale perspective, on significance of EC/ENT exceedances, bacterial sources, and pathogen occurrence.

INTRODUCTION

Water quality of all Great Lakes beaches is defined by concentrations of fecal indicator bacteria (FIB)—typically *Escherichia coli* (EC)—although enterococci (ENT) are also acceptable.[1] FIB concentrations at beaches are influenced by a complex array of variables and have been successfully predicted based on statistical models of local measurements (e.g., debris, algae, bird droppings, number of birds, turbidity) and locally measured hydrometeorological variables.[2] Beach hydrometeorological processes that influence FIB include flow and sediment transport from river watersheds, creeks, and storm drains near or on the beach; runoff from the beach catchment (the area that contributes water directly to the beach during rainfall events); nearshore groundwater flow; long-shore currents; interaction between nearshore and open lake currents; water levels; and wave action. FIB may be entrapped and/or grow in beach sands[3] and are subject to movement to shallow groundwater[4] or to resuspension during appropriate (e.g., high wave) conditions.[5,6] Additionally, FIB are susceptible to mortality from solar irradiation.[7] Sources of FIB may be point (rivers, storm drains) or nonpoint (e.g., debris, algae, bird fecal waste on the beach; overland runoff). Regardless of these recent advancements in our understanding of factors influencing Great Lakes beach microbiological water quality locally, most studies have been performed at, and models developed for, only a single beach.

There is some recent evidence that beach EC concentrations may be influenced on a regional (~40 km) basis.[8−10] The Great Lakes are often referred to as inland seas, as their hydrodynamics are more like those of marine settings and vary among the lakes.[11] Different areas of the Great Lakes experience different climatology.[12] Great Lakes beaches exhibit significant natural variation in geomorphology and material composition, ranging from open dunes, to sand and gravel, or cobble beaches,[13] with varying groundwater tables,[14] and are susceptible to varying degrees of anthropogenic perturbation, including beach sand augmentation, beach grooming, offshore infrastructure such as jetties or piers, and catchment modifications that come with development, such as increased impervious surfaces. Despite these differences, there has been no evaluation of the relative influence of variables that may vary regionally, as opposed to locally, on FIB concentrations at Great Lakes beaches.

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Likewise, our understanding of pathogen occurrence, sources, and association with FIB is based on studies conducted primarily at individual beaches. Recreational water quality criteria were designed based on beach epidemiological studies that showed a link between FIB concentrations and human health.[1] However, there is a widely acknowledged variable relationship between FIB and the concentrations in water of individual disease-causing bacteria, viruses, or protozoa.[15−17] Pathogens have been detected at Great Lakes beaches,[18−22] in gull feces,[23] associated with the nuisance alga *Cladophora*,[24] and in beach sand.[3] Some pathogens, such as the skin-associated *Staphylococcus aureus*, would not be expected to be associated with fecal bacteria[25] and therefore warrant specific study.

In this study we sought to address how FIB concentrations, genes indicating bacterial pathogens commonly associated with swimming-associated disease, and microbial source tracking genes differ across the Great Lakes and among beaches in similar Great Lakes settings but having different local influences. We show that adjacent Great Lakes beaches share regional geographic and hydrometeorological characteristics, FIB temporal patterns, and bacterial pathogen types and frequency of occurrence but that FIB concentrations are more a result of local geographic setting. Results of this study provide insight into the variable role of beach-scale and larger-scale factors that may influence FIB and pathogens at Great Lakes beaches and suggest numerous avenues for future research.

METHODS AND MATERIALS

Site Description. In consultation with local beach managers, who performed the sample collection and recorded daily data, 12 representative beaches were selected (Supporting Information Figure S1): 5 on Lake Michigan in Wisconsin (LM1−LM3) and Michigan (LM4 and LM5), 5 on Lake Erie in Ohio (LE1 and LE2) and New York (LE3−LE5), and 2 on Lake Huron in Michigan (LH1 and LH2). Within these regional shoreline segments with similar beach orientation and overall geographic setting, adjacent beaches were chosen to have variable local land cover and anthropogenic features. National Hydrography Dataset version 1 or 2 catchments[26,27] were selected that contained each beach sampling site. Adjacent watersheds were defined (ArcMap 10.0, ESRI, Redlands, CA) as those occurring on either side of the beach center and were determined by selecting catchments that corresponded to the upstream area (Supporting Information Figure S2). Watershed and catchment delineations were overlaid with the appropriate data sets to determine percent land cover types,[28] population,[29] and soil particle size.[30] Additional environmental variables included beach-specific, weather-related, hydrologic, and sanitary survey data from various sources (Supporting Information Table S1).

Sample Collection and Processing. From June−September 2010, 273 beach water and 22 tributary samples were collected by cooperating beach managers under varying environmental conditions and received at the USGS Michigan Laboratory following overnight shipping on ice. Membrane filtration was used to enrich for target bacteria from a 100 mL beach water sample (Supporting Information Table S2). All growth on the membrane filter was collected by 15 min agitation in phosphate buffered saline (PBS; 10 mL), followed by centrifugation and pellet resuspension in 1 mL of 20% glycerol/0.5 × PBS, then stored at −70 °C until analysis. Details are in the Supporting Information.

Assays for Pathogen Gene Detection. DNA was extracted from 100−400 μL of the glycerol stock using the DNeasy Blood and Tissue DNA extraction kit (Qiagen, Germantown, MD). For optimum recovery, samples were incubated at 4 °C for 15 min immediately following ethanol addition and before filtration. Extracted DNA was stored at −20 °C. Presence/absence polymerase chain reaction (PCR) analysis (see Supporting Information for references and assay conditions) was used to detect virulence or species-specific genes (hereafter, pathogen genes) associated with Shiga-toxin producing EC (STEC), *Shigella* spp., *Salmonella* spp., *Staphyococcus aureus*, and the *mecA* gene associated with methicillin resistant staphylococci, and the 16S rDNA gene for *Campylobacter jejuni* and *coli* (Supporting Information Tables S2 and S3). Although not quantitative, a positive presence/absence result indicates it is likely that one viable target organism was present in the 100 mL water sample. Quality assurance details are in the Supporting Information.

Microbial Source Tracking Markers. Filters representing 100 mL of beach water were immediately frozen at −70 °C, shipped to the USGS Columbus, OH, laboratory and preserved at −70 °C until analysis. DNA extraction (DNA-EZ, GeneRite, North Brunswick, NJ) was according to manufacturer's instructions, except that no prefilter was used and the final elution volume was 200 μL. Quantitative polymerase chain reaction (qPCR) assays were conducted for general *Bacteroides* (AllBac),[31] human (HF183 and HuBac)[31,32] or ruminant-associated *Bacteroides* (BoBac),[31] and a gull-associated marker from *Catellicoccus marimammalium* (Gull2).[33] All qPCR assays were performed as described in the citations, with the exception of Gull2, in which a double-quenched probe was used. The probe, labeled with an internal ZEN Quencher and an Iowa Black Quencher (Integrated DNA Technologies, Coralville, IA) was designed to reduce background and increase qPCR signal. qPCR quality assurance and details of copy number determination are in the Supporting Information.

Statistical Analysis. Statistical analyses were performed in Systat 13 (Systat Software, Chicago, IL). Water quality parameters not normally distributed; all were $\log_{10}$-transformed. Land cover was evaluated as the arc-sine[34] transformation of percent of land cover. Gene detections were evaluated as arc-sine transformed proportions or frequencies of detection or on log10 of quantitative data. All statistical analyses were performed on transformed data unless otherwise noted. Typically, data were examined by Pearson correlation with Bonferroni adjusted probabilities, and discriminant analysis was performed to identify variables that discriminated among various groupings of the data. Kruskal−Wallis (multiple comparison) or Mann−Whitney U tests (paired comparisons) were also performed to evaluate the significance of variable difference across groupings of the data. Virtual Beach software[35] was used to conduct multiple linear regression of transformed variables to identify factors associated with beach seasonal geometric mean concentrations of FIB.

RESULTS AND DISCUSSION

Regional and Local Variation in Beach Setting. Environmental variables varied among regional beach groups (Table 1). For example, New York Lake Erie beaches had the greatest ranges of water level variation over 24 h (Table 1). Lake Erie is susceptible to large oscillating standing waves (seiches).[36] In contrast, average wave height, a function of beach slope, was greatest for LM4 and LM5 beaches and also

Table 1. Selected Characteristics of the Twelve Studied Great Lakes Beaches[a]

beach groups				hydrometeorology				watershed characteristics				beach characteristics							
beach ID	lake	state	distance between beaches (km)	beach orientation (degrees from North)	water direction (to) parallel to beach and average velocity (m/s)	24 h water level difference (m, min/max)	average wave height (m)	average cloud cover (%)	suspended sediment yield: average both watersheds (kg/km^2·y)	dominant watershed land cover (%)[b]	distance (km) and area (km^2) South or West watershed	distance (km) and area (km^2) North or East watershed	catchment urban land cover (%)	catchment forest land cover (%)	Log10 catchment population (no./km^2)	catchment area (km^2)	catchment impervious surface (%)	drain or creek on beach	average debris category (1–3)	Log10 average number of gulls on beach
LM2	Michigan	Wisconsin	--	135	N/0.06	−0.12/0.06	0.24	26	4.9	Ag (49)	7.1/12	4.2/113	5	7	2.05	7.28	1	Y	1.1	0.38
LM3	Michigan	Wisconsin	40.3	90	N/0.06	−0.19/0.06	0.19	32	5.3	Ag (68)	7.7/36	6.9/24	14	6	1.15	2.33	2	N	1.1	0.41
LM1	Michigan	Wisconsin	19.3	90	N/0.05	−0.16/0.10	0.12	39	5.3	Ag (71)	8.2/64	1.6/1343	83	0.3	3.87	6.88	43	Y	1.8	1.38
LM4	Michigan	Michigan	--	250	N/0.07	−0.05/0.06	0.44	34	4.5	MIX	7.2/14338	6.1/27	17	28	2.04	2.59	5	N	1.9	0.55
LM5	Michigan	Michigan	3.2	232	N/0.04	−0.06/0.07	0.45	34	4.5	Ag (50)	--	0.8/14338	18	50	3.01	3.70	5	N	2.3	0.92
LE1	Erie	Ohio	--	324	E/0.03	−0.11/0.23	0.23	60	5.6	Urb (98)	8.5/206	0.3/6	95	3	4.73	28.51	41	Y	1.3	0.65
LE2	Erie	Ohio	19.3	344	E/0.02	−0.10/0.20	0.32	61	5.7	Urb (44)	8.6/779	3.22/2215	97	0.6	4.97	25.99	44	Y	1.4	1.84
LE4	Erie	New York	--	314	E/0.04	−0.20/0.16	0.24	47	5.3	FOR(51)	7.9/31	0.1/24	81	39	2.78	3.59	3	Y	1.5	1.31
LE3	Erie	New York	3.1	323	E/0.04	−0.23/0.13	0.16	41	5.3	FOR(47)	7.9/1456	0.1/31	25	10	1.90	0.13	21	Y	1.7	1.72
LE5	Erie	New York	20.9	271	S/0.01	−0.23/0.13	0.19	64	5.4	Urb (38)	8.3/9	0.4/22	66	8	2.10	1.21	29	Y	1.1	1.11
LH1	Huron	Michigan	--	41	N/0.02	−0.06/0.06	0.07	38	4.6	MIX	7.7/7	2.9/4	13	10	2.02	3.01	2	N	2.0	0.97
LH2	Huron	Michigan	10.3	83	S/0.04	−0.06/0.06	0.09	37	4.6	FOR (35)	6.8/12	0.1/393	25	9	1.86	2.33	7	Y	2.0	0.17

[a]All data sources and parameters are defined in the Supporting Information; standard deviations for averages in Table S8. [b]AG, agriculture; FOR, forest; URB, urban.

varied with beach orientation to the dominant S−SW winds typical of the Great Lakes during the summer.[12] Percent cloud cover, a variable that may influence survival of FIB[7] was greatest at Lake Erie beaches, and rainfall is historically greater at Lake Erie stations than at Lake Huron or Lake Michigan stations near our selected beaches.[12] Watershed suspended sediment yield was greatest for the Ohio Lake Erie beaches (LE1 and LE2). The absolute value of water velocity parallel to the beach, average 24 h water-level difference, average wave height, average cloud cover, average suspended sediment yield of adjacent watersheds, and average debris category were significantly different among the beach groups and were highly effective at group discrimination (discriminant analysis, Wilk's lambda <0.0005, p = 0.007).

Within regional beach groups, beaches also varied with respect to local variables such as proximity to, size of, and land cover in adjacent rivers; presence of drains or creeks on the beach; catchment area, land cover, population, and impervious surface; and with respect to beach observations such as average gull numbers (Table 1). Using data on beach sources and conditions, and beach, catchment, and watershed characteristics (Supporting Information Table S1, section B), discriminant analysis did not identify local variables unique to regional beach groups (Wilk's lambda 0.00, p = 0.123), as expected because these features varied within beach groups.

Regional and Local Influence on Fecal Indicator Bacteria Densities and Patterns. Beach groups generally exhibited strikingly similar temporal fluctuations of EC (Figure 1) and ENT (Supporting Information Figure S3) throughout the sampling season. Beach LE1 has a series of offshore structures designed to modify wave impacts that may influence FIB patterns observed for that beach. Virtual Beach models (Virtual Beach,[35] multiple least-squares (MLR) regression; Supporting Information Table S4) all had adjusted R^2 > 0.59. These relatively high values are likely influenced by having few (17−27) observations. Models derived from multiple years of data (often >100 observations) typically exhibit lower R^2 values.[9,10,37,38] Models did not appear to offer a set of common variables for beaches within groups (Supporting Information Table S4), but hydrometeorological variables related to wind speed or direction, wave height, and wave direction were significant in every model. Wave-related variables were singularly significant in some groups. Wave-height alone was significantly correlated with EC at LM4 (0.723, p = 0.035) and marginally (0.685, p = 0.096) at LM5. Onshore wave direction was associated with greater EC concentrations in groups LE1 and LE2, LM4 and LM5, and LM1−LM3 (Mann−Whitney U, p = 0.009, 0.001, and 0.054, respectively) and with greater ENT concentrations at LM1−LM3 (p = 0.009). Wave height, a function of beach slope, is governed by regional factors such as the underlying geology, as well as orientation facing prevailing winds,[14,39] and was greatest for the LM4 and LM5 beaches, located in part of the largest freshwater dune system in the world.[39] Waves may wash debris or bird droppings from the shore, suspend bacteria-laden sands, or mobilize the transport of bacteria.[4] Increased turbidity associated with waves may protect bacteria from solar radiation.[7] Similar temporal patterns and spatial correlation between EC concentrations, for geographically proximal beaches, have been noted previously[8−10] for beaches in Lake Michigan. In those studies, predictive models for EC concentrations for regional groups of beaches using just a few variables (e.g., wave height, wind direction, barometric pressure, rainfall) had R^2 values in the

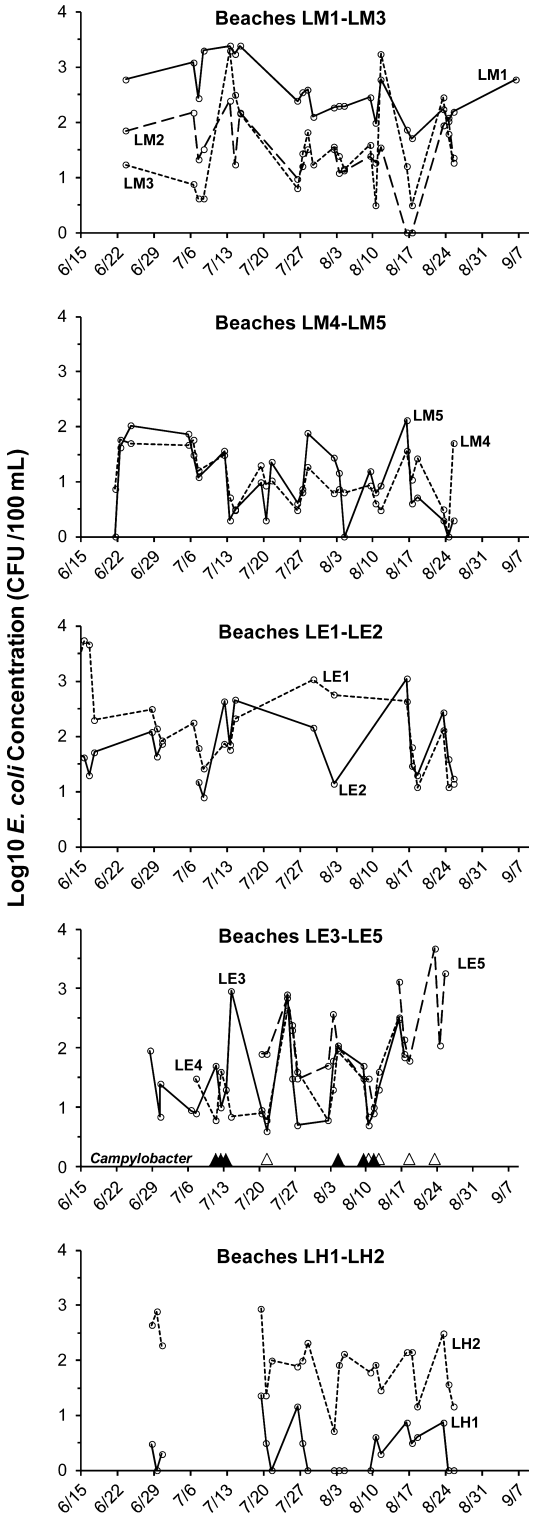

Figure 1. Patterns of *E. coli* concentrations by beach group and date. For beach group LE3−LE5, triangles denote detection of the *Campylobacter jejuni/coli* gene: (open triangles) detection at only one beach; (closed triangles) detection at two or three beaches.

range of those for individual beaches, consistent with our findings.

Regardless of these more regional-scale influences, local factors related to beach sources or conditions (Supporting Information Tables S1 and S4) were also explanatory model variables for either EC or ENT in all beach groups except LE3−LE5. EC or ENT concentrations were dramatically greater at LM1, LE1, LE5, and LH2 than at other beaches in their respective groups (Table 2, Figures 1 and S3), and these were the beaches where recreational water quality criteria for EC or ENT, or both, was exceeded (Table 2). USEPA[1] recommends an EC geometric mean (GM) of 126 CFU/100 mL and, in addition, that fewer than 10% of samples should have an EC value exceeding 410 CFU/100 mL, the latter designated the statistical threshold value (STV). Alternatively, the values for ENT are the following: GM, 35 CFU/100 mL and STV, 130 CFU/100 mL. Discriminant analysis using all available local variables was not conclusive in identifying variables that discriminated between these four beaches and those with no EC or ENT exceedances (Wilk's lambda 0.252, $p = 0.904$); however, catchment impervious surface and percent urban land cover varied notably between these two beach categories, and these two variables were significantly different between beaches exceeding or not exceeding just the EC criteria (Mann−Whitney U, all $p < 0.05$). Therefore, for further analysis, beaches were grouped into those with greater than 20% impervious surface (CATCH_IMP > 20%) or with greater than 20% urban land cover (CATCH_URB > 20%), based on apparent breaks in our data set (Table 1). Because drains or creeks have previously been noted to influence Great Lakes beach FIB,[10] DRAIN/CREEK was also evaluated. Concentrations of EC and ENT were significantly greater at DRAIN/CREEK, CATCH_IMP > 20%, or CATCH_URB > 20% beaches (Mann−Whitney U, $p < 0.003$ for all tests, $n = 273$). Beaches that never exceeded any GM or STV for EC or ENT had no drain or creek and <20% impervious surface or urban land cover in the catchment (Table 2), thus indicating catchment-scale features as primary factors influencing FIB concentrations. Creeks are natural features of some beach catchments; in contrast, drains are anthropogenic constructs, often following natural flow patterns, but designed to route water more quickly from urban settings. Our catchment delineations are not perfect in that storm drains may have catchment basins that extend outside the natural catchment area. Nevertheless, rainfall runoff[40] is clearly understood to influence beach microbiological water quality, and the catchment is the most immediate source of rainfall runoff to the beach.

Some beaches were also influenced by having river mouths very close to one side of the beach, a factor documented to influence beach water quality in the Great Lakes and elsewhere.[41−43] For example, in the LE3−LE5 group, beach LE5, with the greatest EC and ENT geometric means, has a river mouth very near the north side of the beach (Table 1), and Supporting Information Figure S4 shows that EC concentrations increased when water direction was to the south. In contrast, the adjacent LE3 and LE4 have nearby river mouths on the east, but flow direction was typically to the east, moving river water away from these beaches (Figure S4). Among the four beaches with EC and ENT exceedances, LE1, LE5, and LH2 all showed interaction between lake current direction and proximal rivers on EC concentrations, but LM1 did not (Figure S4) because currents virtually never brought water south (from the proximal river to the north) at that beach. In addition, rainfall and current flow direction parallel to the beach are also linked at some beaches (notably again at LE5 and LH2, Supporting Information Figure S5), but not at others, likely due in part to different orientation to the humid southerly air masses associated with most summer precipitation in the Great Lakes.[12] Thus, in the larger framework of beach

Table 2. Summary Data for Fecal Indicator Bacteria Concentrations, General *Bacteroides* (AllBac gene) Copy Number, and Bacterial Pathogen Gene and MST Marker Detection Frequencies for 12 Great Lakes Beaches, June−September 2010[a]

							gene detection frequency												
							source tracking			Shiga-toxin producing *E. coli*				*Shigella*	*Salmonella*	Methicillin resistance and *Staphylococcus aureus*			*Campylobacter jejuni or coli*
beach ID	number of samples	*E. coli* geometric mean (CFU/100 mL)	exceedance of *E. coli* STV[b]	enterococci geometric mean (CFU/100 mL)	exceedance of *Enterococcus* STV	Log10 geometric mean of general *Bacteroides* (AllBac) gene copy number/100 mL	QHF183 (human)	BoBac (ruminant)	Gull2	*eaeA*	*stx2*	*stxI*	rfb_{O157}	*ipaH*	*invA*	*spvC*	*mecA*	*femA*	*mecA* + *femA*	16S rDNA
LM2	23	29	NO	12	YES	6.0 (11/8)[d]	0.18	0.00	0.13	0.70	0.00	0.09	0.09	0.09	0.00	0.04	0.26	0.22	0.17	0.04
LM3	23	32	NO	9	NO	5.1 (12/7)	0.17	0.08	0.57	0.83	0.00	0.17	0.17	0.09	0.04	0.13	0.09	0.00	0.00	0.00
LM1	23	**306**[c]	**YES**	**55**	**YES**	6.4 (12/9)	0.50	0.08	0.44	0.88	0.13	0.13	0.17	0.17	0.04	0.21	0.04	0.04	0.04	0.00
LM4	27	10	NO	10	NO	3.8 (13/10)	0.15	0.23	0.40	0.70	0.07	0.07	0.07	0.00	0.00	0.11	0.44	0.04	0.04	0.04
LM5	27	8	NO	12	NO	4.1 (13/10)	0.08	0.00	0.50	0.74	0.07	0.19	0.07	0.04	0.00	0.07	0.33	0.19	0.15	0.00
LE1	22	**140**	**YES**	30	YES	3.9 (12/9)	0.33	0.25	0.56	0.96	0.17	0.17	0.13	0.09	0.04	0.04	0.26	0.30	0.22	0.09
LE2	23	68	YES	17	NO	4.2 (14/11)	0.29	0.21	0.64	1.00	0.14	0.14	0.05	0.18	0.09	0.05	0.14	0.32	0.09	0.09
LE4	23	34	NO	14	YES	4.4 (15/13)	0.27	0.00	0.31	0.87	0.09	0.04	0.04	0.04	0.00	0.13	0.48	0.43	0.30	0.30
LE3	23	35	NO	9	YES	4.6 (15/13)	0.40	0.00	0.17	0.83	0.17	0.00	0.09	0.00	0.04	0.13	0.30	0.17	0.09	0.30
LE5	17	**144**	**YES**	**69**	**YES**	4.8 (8/5)	0.25	0.25	0.60	1.00	0.06	0.24	0.24	0.12	0.24	0.53	0.18	0.24	0.00	0.29
LH1	21	2	NO	1	NO	4.4 (10/6)	0.20	0.30	0.17	0.43	0.00	0.10	0.10	0.00	0.05	0.00	0.38	0.00	0.00	0.05
LH2	21	88	YES	**93**	**YES**	4.3 (10/9)	0.10	0.10	0.11	0.95	0.38	0.33	0.24	0.00	0.24	0.52	0.38	0.05	0.00	0.14

[a]Genes described in the Supporting Information. [b]STV, statistical threshold value.[1] [c]Bold highlight indicates values that exceed Recreational Water Quality Criteria.[1] Both the GM and STV values must be exceeded. [d]Number of samples (AllBac, QHF183, or BoBac/Gull2).

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orientation to weather patterns and lake currents, proximity to rivers is a local geographic variable, that, in addition to catchment features, may influence FIB concentrations.

Regional and Local Variation in Microbial Source Tracking Markers. The AllBac (general *Bacteroides*) gene was detected in all but 10 samples. The number of AllBac copies/100 mL (CN) was significantly greatest within the LM1−LM3 group (Kruskal−Wallis, $p < 0.005$; Table 2), but we were unable to identify any variable correlated with this difference. Likewise, there was no significant correlation between AllBac CN and any local variable within any group (all $p > 0.122$). AllBac CN was greater at DRAIN/CREEK beaches (Mann−Whitney U, $p = 0.020$; Supporting Information Table S5), but there was no effect of river proximity on AllBac CN as there was for EC concentration. There was no universal correlation between AllBac copy number and either EC or ENT concentration ($n = 145$, $p > 0.3$ for both). AllBac CN was correlated with EC (but not ENT) concentration only in the LM1−LM3 group (0.432, $p = 0.029$, $n = 35$). The relation between AllBac copy numbers and FIB has been variable in other studies.[44−47] Gentry et al.[48] observed spatial dependency in correlation between AllBac, BoBac, and EC loads, river flow, and turbidity at a progression of upstream to downstream locations within a watershed. We did not determine loading rates for these parameters, which might provide different results, but which are challenging to obtain for beaches.[49] In our study, AllBac and EC concentrations were not linked, and the BoBac marker (see below) was not detected at every beach. In the present study AllBac was not correlated with EC or ENT in part because (1) AllBac was not as influenced by catchment features or proximal rivers as were EC or ENT and (2) there was strong geographic variation in AllBac CN.

Microbial source tracking (MST) markers indicated that no beach was susceptible to only one source and that sources varied not only by beach within regional groups (Table 2) but also by day at individual beaches (Supporting Information Table S6). There were 145 HF183 or HuBac (human) and BoBac (ruminant) and 108 Gull2 samples. The HF183 and Gull2 markers were detected at every beach, but BoBac was not (Table 2). HuBac was detected in only 63% of samples positive for HF183 (and 2 where HF183 was not detected) and was not further studied. HF183 was detected in 25%, BoBac in 12%, and Gull2 in 38% of tested samples. For each of these genes, there was no significant difference in the EC or ENT concentration (Mann−Whitney U, $p > 0.2$ for all, $n = 145$ or 108) between samples having or not having detections. There were too few detections to meaningfully evaluate the correlation between EC, ENT, and MST marker detections at individual beaches. It was rare to detect more than one MST marker in a sample (Supporting Information Table S6). Of the 145 samples tested for both HF183 and BoBac, only 5 had both markers, while 43 had one, and 97 had none. Likewise, of the 108 samples tested for all three markers, only 3 had all three present. Because of this extreme variability and because only a subset of samples were tested for MST markers, no overall or within-beach-group relationships with environmental variables could be established. Nevertheless, we did establish that although neither the frequency of detection nor CN of the Gull2 marker was correlated with gull numbers ($n = 108$; $p > 0.487$ for both tests), HF183 and Gull2 (marginally) were greater at CATCH_URB > 20% beaches ($p = 0.003$ and 0.091, respectively; Supporting Information Table S5). Gull numbers were positively correlated with percent catchment (but not watershed) urban land cover (Pearson $r = 0.625$, $p = 0.03$) suggesting that urban and gull sources of microbiological contamination may be linked and that it may be difficult to separate these sources at such beaches. Gulls are recognized as a problem in urban areas,[50] and the Gull2 marker decreased when gulls were removed from a Lake Michigan beach.[51] We also established that the BoBac marker was not correlated (all $p > 0.230$) with any measure (Supporting Information Table S1) of agricultural influence. The BoBac marker detects numerous ruminant sources and, at lower levels, other animals.[52] Over the entire data set, the average number of copies/100 mL (±SD) in samples with detections was 3797 ± 8938 for HF183 (14% of AllBac), 2950 ± 5151 for BoBac (7%), and 165 ± 400 for Gull2 (2%), which is far lower than the AllBac concentrations (Table 2). Schriewer et al.[44] suggested that low proportions of source-tracking indicators with respect to AllBac may indicate many other sources of fecal bacteria in surface waters, but differential survival and distance from source may also influence these proportions.[47]

Regional and Local Variation in Pathogen Gene Markers. *Relation Between FIB, MST Markers, and Pathogen Genes.* Exceedance of the beach action value (BAV), but not of the GM/STV criteria, was associated with increased numbers of pathogen gene detections at beaches. Using the GM and STV for the samples analyzed, three beaches exceeded the EC GM/STV and three, the ENT GM/STV (Table 2). There was no difference in the total number of pathogen genes detected, or their detection frequency, at beaches that met or exceeded either the EC GM/STV or the ENT GM/STV criteria (Mann−Whitney U, $p > 0.261$ for both). However, the USEPA also offers a single-sample maximum BAV of 235 CFU/100 mL for EC and 70 CFU/100 mL for ENT as a precautionary tool for beach notification. There were 45 single samples (of 273) that exceeded the EC BAV, and 57 that exceeded the ENT BAV. Significantly more total genes were detected in samples that exceeded a BAV (Mann−Whitney U, $p = 0.003$ for EC; $p < 0.0005$ for ENT). There was no correlation between AllBac CN and total pathogen genes in the 145 samples tested, or when tested within beach groups. EC and ENT concentrations were correlated (0.729, $p < 0.0005$, $n = 273$), and both FIB were correlated with detection frequency of the STEC genes *eaeA*, *stx2*, rfb_{O157}, and the *Salmonella spvC* gene (Supporting Information Table S7), while the remaining genes were not correlated with FIB concentrations. AllBac, HF183, BoBac, or Gull2 geometric mean CN was not correlated with any gene detection frequency at any beach (all $p > 0.332$). The relationship between FIB concentrations or MST marker CN and specific pathogens is variable.[44,53,54] Wu et al.[17] report that sample size and number of samples positive for pathogens greatly influence the ability to establish a relationship between FIB and pathogens. Our study indicates that geographic variation also influences this relationship for Great Lakes beaches.

Pathogens at Beach Groups. Pathogen types varied among beach groups (Table 2, Figure 2). The *invA* gene was absent at LM4 and LM5, *ipaH* was absent at LH1 and LH2, *femA* was more consistently prevalent at LE beaches than at beaches on other lakes, and the *Campylobacter jejuni/coli* 16SrDNA gene notably occurred in about 30% of the samples at each beach in the LE3−LE5 group. Principal components analysis using the frequency of detection of each gene broadly grouped adjacent beaches (Figure 2) with two exceptions (discussed below). There were too few pathogen gene detections to establish

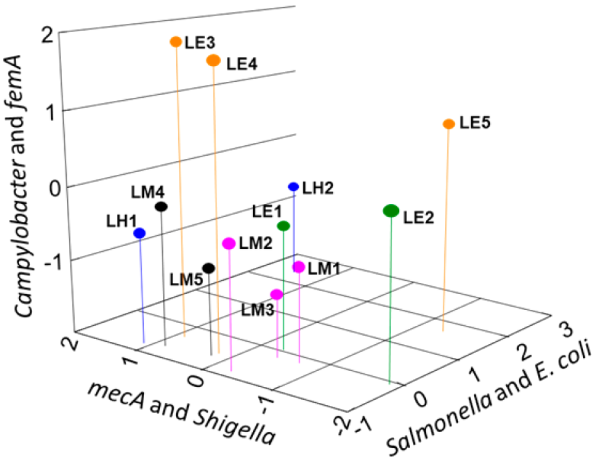

Figure 2. Diagram depicting results of principal components analysis of beaches by all pathogen genes. The genes or bacteria associated with each principal component are identified as axis labels. The three principal components identified a total of 85% of the variance in the data. Principal component 1 explained 43% of the variance and was positively associated (component loadings) with the rfb_{O157} (0.86), *invA* (0.95), *spvC* (0.91), and *stx1* (0.82) genes. Principal component 2 explained 22% of the variance and was positively associated with the *mecA* gene (0.743) and negatively associated with the *ipaH* gene (−0.96). Principal component 3 explained 20% of the variance and was positively correlated with the *femA* (0.825) and *Campylobacter* (0.835) genes.

relationships with environmental variables at individual beaches or within beach groups

Campylobacter. The most pronounced pathogen signature was that of *Campylobacter* (Table 2). Even more remarkable is the similar pattern of temporal occurrence of *Campylobacter* genes at beaches LE3−LE5 (Figure 1), encompassing ∼21 km of shoreline. At beaches LE3−LE5, the 19 *Campylobacter* detections (in 63 samples) were not correlated with any environmental factor nor with EC or ENT (Pearson correlation, all $p > 0.6$). However, *Campylobacter* were more frequently detected overall at DRAIN/CREEK or CATCH_IMP > 20% beaches (Supporting Information Table S5). There was no correlation between *Campylobacter* detections and detections of other genes ($n = 273$) and likewise, no correlation with any of the MST markers ($n = 145$ or 108), even at beaches LE3−LE5 ($n = 38$). The tributary to the north of LE5 was sampled on one date, but *Campylobacter* was not detected on that date. The presumed source of *Campylobacter* is birds,[55] *Campylobacter* is present in gull feces in the Great Lakes,[23] and the frequency of detection of *Campylobacter* by qPCR decreased following gull control at a Lake Michigan beach.[51] Gull numbers at our study beaches were not associated with *Campylobacter* detections, but waterfowl abundance does not necessarily indicate predominant bird source.[56] *Campylobacter jejuni* isolated from water or wildlife may be associated with human infection.[56,57] Our results are suggestive of an undetermined *Campylobacter* source or transport process at a scale larger than the individual beach, and to our knowledge, ours is the first documentation of regional variability in *Campylobacter* detections in Great Lakes beach water.

Salmonella and Shiga-toxin Producing E. coli. The *Salmonella* genes (*invA* and *spvC*), as well as the STEC *stx2* gene, and the *eaeA* gene, found in most STEC, were most frequently detected at DRAIN/CREEK and/or CATCH_IMP > 20% beaches (Mann−Whitney U, Supporting Information Table S5). The *stx2* gene is present in STEC from multiple sources (pigeons, sheep, cattle, pigs), with varying implications for human health. *Salmonella* have been detected in gull feces.[23] The *Salmonella spvC* gene, and three of the four STEC genes, were correlated with FIB concentration (Supporting Information Table S7), which is reasonable as STEC might be expected to follow EC patterns to some extent, and *Salmonella* is closely related to EC.

Beaches LE5 and LH2 were characterized by having the highest frequencies of detection of both *Salmonella* genes, and of the *stx1* and rfb_{O157} genes, separating them from their respective beach groups (Table 2 and Figure 2). Beach LH2 also had the most frequent detection of the *stx2* gene. Although these beaches had very little in common, being on different lakes, and having very different watershed and beach characteristics (Table 1), both beaches have a drain or creek on the beach and both have a proximal river that influences EC concentrations, as described previously (Supporting Information Figure S4). The tributary to the north of LH2, a large rural river, was sampled 15 times. *Stx2* was detected in 36%, rfb_{O157} in 13%, *stx1* in 7%, *invA* in 20%, and *spvC* in 60% of those samples and all except the *invA* gene were detected in the single LE5 tributary sample. These results implicate runoff via drains, creeks, rivers, and impervious surfaces (factors that also influenced EC) in the detection of some STEC carrying *stx2* and rfb_{O157} and of *Salmonella.* In contrast, the STEC *stx1* gene, most frequently detected at beaches LE5 and LH2, was not associated with FIB concentrations or with the factors that influence FIB (or with any other factor at these two beaches). STEC carrying the *stx1* gene are generally associated with cattle (or wildlife such as deer).[58] These results suggest a different source and possibly a different delivery pathway, for STEC carrying the *stx1* gene.

Shigella and Staphylococcus. The *ipaH* gene, indicative of *Shigella* (human source) or enteroinvasive EC (closely related to *Shigella*, also human source, rare in U.S.),[59] was detected infrequently, but beaches LM1, LE2, and LE5 had detection frequencies exceeding 10%. The most notable characteristic of these beaches is their urban nature (Table 1), and the *ipaH* gene was detected more frequently at CATCH_URB > 20% beaches (Supporting Information Table S5) and was not correlated with FIB concentrations (Supporting Information Table S7). The *ipaH* gene was detected in only 3 of 22 tributary samples, and only once concurrently with a beach detection. *Shigella,* like other fecal pathogens, could arise from swimmers themselves.[60]

Staphylococcus are considered derived from swimmers' skin rather than fecal sources,[25] and as expected, there was no association between the *femA* gene and EC or ENT concentrations (Supporting Information Table S7). Methicillin-resistant *S. aureus* (MRSA) have both the *femA* and *mecA* (methicillin resistance) genes. The *mecA* gene may also occur in other staphylococci. The *mecA* gene was detected at all 12 beaches, only two beaches had detection frequencies less than 10%, and frequencies of detection at paired beaches were generally similar (Table 2). No environmental factor was clearly associated with *mecA* frequency of detection, but with only two beaches with low frequencies of detection, there may not have been sufficient variability in the data to evaluate influencing factors. *MecA* (1/22) and *femA* (2/22) were detected infrequently in tributary samples. The *mecA* gene was more frequent at CATCH_URB > 20% beaches, but *femA* was most associated with DRAIN/CREEK and CATCH_IMP > 20% beaches (Supporting Information Table S5). Potential MRSA (both *mecA* and *femA*

detected in the same sample) occurred at a frequency of >10% at four beaches (Table 2). As samples in our study were usually collected early in the day, and on weekdays, the number of people in the water could not be associated with *femA* or MRSA detections in our 273-sample data set. Taken together, an urban influence appears to play a part in *Shigella* and *Staphylococcus* occurrence. Overall, our results suggest regional variation in bacterial pathogens at Great Lakes beaches, and for the *stx1* gene, *Campylobacter*, and *Staphylococcus* possibly different sources or transport pathways than for EC or ENT.

In conclusion, ours is the first study to compare FIB concentrations, MST markers, and genes indicating bacterial pathogens across Great Lakes beaches, using regional and local geographic features to assess variables that may influence microbiological water quality. Ours is the first effort, to our knowledge, to apply a hydrologically meaningful "watershed" (the catchment) to beach studies, and we found that catchment variables such as urban land cover, impervious surface, and the presence of drains or creeks on beaches influenced FIB and general *Bacteroides* concentrations, the presence of *Bacteroides* human and gull source-tracking markers, and the occurrence of most pathogen genes. Additionally, local proximity of rivers adjacent to beaches, in a complex interaction with regional variables such as lake currents and rainfall patterns, also influenced exceedance of recreational water quality standards, and detections of *Salmonella* and STEC. In contrast to these beach-specific findings, we noted similar temporal fluctuations of FIB within groups of adjacent beaches, and similar temporal patterns of *Campylobacter* gene detections within one group. We could not resolve what causes these consistent regional-scale patterns, but our data indicate that beaches exist in a geographic hierarchy whereby regional variables appear to dominate the day-to-day fluctuations of FIB, and of some pathogens, but concentration of FIB is aggravated by greater proximity to, and enhanced transport of, local sources, through features such as impervious surfaces, creeks, drains, or nearby rivers. Some of these aggravating factors (e.g., storm drains, bird numbers) may be amenable to remediation. Recognizing the roles of unique regional geographic and hydrometeorological conditions, as well as local conditions, will be important to beach management, restoration, and the design of future studies. Additional regional beach features that we did not address, such as details of beach geomorphology, beach sand type, the role of hydrometeorology on sand resuspension at beaches of different slope and orientation to prevailing winds, and the role of shallow groundwater beneath beaches, are in need of investigation.

By studying multiple Great Lakes beaches, we obtained new insights that could not be obtained from studies at single beaches. Exceedance of the newly recommended[1] recreational water quality criteria was not associated with increased numbers of pathogen gene detections at beaches. Detections of genes for EC-related pathogens were related to EC concentration, but *Campylobacter* and *Staphylococcus aureus* genes were not. Total *Bacteroides* concentrations varied among regional beach groups, no beach was susceptible to a single source, and source-markers varied daily. Our study is limited in the types of pathogen genes studied, in the number of pathogen genes detected, and by the absence of quantitative data for pathogens. Nevertheless, our study demonstrates the complications of evaluating FIB, pathogens, and sources at beaches, where rapid variation in hydrodynamics, rainfall, and local variables such as bird or swimmer numbers, present a constantly changing source profile, and suggests caution in extrapolating local studies to other beaches.

■ ASSOCIATED CONTENT

Supporting Information

Additional text and tables as mentioned in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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