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Receiver-Operating Characteristics Analysis: A New Approach to Predicting the Presence of Pathogens in Surface Waters

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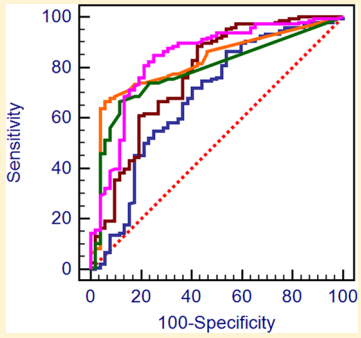
Supporting Information

ABSTRACT: Fecal indicator microbes are used to monitor the public health risks of recreating in surface waters. However, the importance of indicator tests as predictors of waterborne pathogens has been unclear. Numerous studies have also shown that the survival and growth of indicator organisms may depend on location-specific factors that cannot be broadly generalized. We used receiver-operating characteristic (ROC) methods to determine whether fecal indicator species are capable of predicting the presence of *Giardia* and *Cryptosporidium* in fresh surface waters in the Chicago area. We also derived recreational water quality criteria specific to our location with respect to this end point. We considered five fecal indicators: enterococci measured by culture and quantitative polymerase chain reaction (qPCR), *Escherichia coli* measured by culture, somatic coliphage, and F+ coliphage. All fecal indicators were found to predict the presence and absence of protozoan pathogens. The test for enterococci measured by culture was the poorest predictor of the presence of pathogens. The test for enterococci measured by qPCR was the best predictor of the presence of *Giardia*, but not an important predictor of the presence of *Cryptosporidium*. The test for somatic coliphage was a relatively strong predictor of the presence of both pathogens. This analysis supports the use of qPCR-based assays over culture-based assays for predicting the presence of *Giardia* in fresh surface water. Our criteria were optimized for the prediction of the presence of *Giardia* and *Cryptosporidium* in our location and were closely aligned with criteria of the U.S. Environmental Protection Agency derived from epidemiological risk assessment. The ROC approach is flexible and can inform location-specific interpretation of water quality monitoring data and decision making.



INTRODUCTION

Escherichia coli, enterococci, coliform bacteria, and coliphage viruses are prevalent in the intestinal flora of warm-blooded animals and are used to indicate the presence of fecal waste in surface waters.[1] Although these fecal indicators (FIs) are generally not pathogenic to humans, these organisms have been found to predict health risk among swimmers at beaches[2−8] and are an indirect means of determining the safety of water for primary contact recreation.[9,10] The 2012 Recreational Water Quality Criteria (RWQC) of the U.S. Environmental Protection Agency affirmed the use of the FIs *E. coli* and enterococci for beach monitoring and notification purposes.[10]

Outside the context of recognized outbreaks, the microorganisms that account for cases of sporadic gastrointestinal illness in swimmers are rarely known.[11,12] Suspected etiological agents include bacteria[13−15] [e.g., *Campylobacter* spp., *Salmonella* spp. (non-typhoid), *Listeria* spp., and *E. coli* O157:H7], protozoans[14,16] (e.g., *Cryptosporidium* spp. and *Giardia* spp.), and viruses[17] (e.g., adenovirus, norovirus, enteroviruses, and rotaviruses). *Giardia* and *Cryptosporidium*, in particular, are consistently identified as being important etiological agents of gastrointestinal disease outbreaks in U.S. recreational waters[18] and drinking water, as was the case in the 1993 Milwaukee outbreak.[19]

Common acute adverse health outcomes associated with ingestion of water during recreation include diarrhea, vomiting, fever, skin rashes, and respiratory, ear, and eye ailments.[6−8,20] Unlike FIs, pathogens appear intermittently in surface waters at recreation sites, often at concentrations near detection limits.[16,21,22] This may be because pathogens are shed only by infected animals or introduced to recreational waters only following heavy precipitation, but it is also the case that pathogen sample collection and analytical methods have limited sensitivity.[23,24] These may be the reasons for conflicting data regarding the correlation between FIs and pathogens in recreational waters, particularly with respect to the protozoan pathogens *Giardia* and *Cryptosporidium*.[16,21,22] Because the infectious doses of *Cryptosporidium* as well as viral pathogens (such a norovirus) are quite small,[25,26] the presence of pathogens in recreational waters, even infrequently or at low concentrations, is a public health concern.

Understanding relationships between FIs and pathogens in recreational water can help in the assessment of the health risks for specific locations. For example, if selected pathogens are

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recognized to be of concern at a particular location, the thresholds of FIs used to declare recreational advisories can be optimized to reflect potential exposures and health risks posed by pathogens at that location. Site specificity is important because the survival and density of indicator organisms can vary among sites for reasons having little to do with fecal pollution sources.[27−33] Consideration of the presence of a pathogen may aid in the development of site-specific optimization of FI thresholds for protecting health.

Identification of the threshold of a continuous variable, such as FI density, for the optimal prediction of a dichotomous outcome, such as protozoan pathogen presence, is a trade-off between sensitivity and specificity. A sensitive threshold accurately predicts the presence of a pathogen, while a specific threshold accurately predicts the absence of a pathogen. A method for optimizing this trade-off is receiver-operating characteristic (ROC) analysis.[34] There has been some limited interest in using ROC to enhance risk assessment at bathing beaches. Morrison et al. applied this method to predict if surface water FI criteria have been exceeded on the basis of prior day FI densities and precipitation data.[35] Studies have also used the ROC curve to evaluate the discriminatory power of water exposure,[36] food consumption,[36] and indicator density[36,37] with respect to the incidence of swimming-related adverse health outcomes. To the best of our knowledge, only one other study has used ROC analysis to evaluate the relationship between indicator density and the presence of a pathogen. Efstratiou et al. used ROC analysis to determine whether total coliforms, fecal coliforms, and enterococci could predict the presence of *Salmonella* in seawater.[38] However, this study did not analytically derive optimized criterion thresholds, nor did it statistically differentiate between predictive power of indicator tests using ROC analysis.

The objective of this study is to compare several FIs based on their ability to predict the presence of the protozoan pathogens *Giardia* and *Cryptosporidium* in Chicago area surface waters using ROC analysis. The following FIs were considered: *E. coli* (measured by culture), enterococci (measured by culture and qPCR), somatic coliphage, and F+ coliphage. Specifically, we (1) determined whether culture-based measures of *E. coli* and enterococci, somatic coliphage, and F+ coliphage and the qPCR-based measure for enterococci can predict the presence and absence of *Giardia* and *Cryptosporidium*, (2) calculated the optimal thresholds at which FIs predicted the presence and absence of a pathogen, (3) compared pathogen prediction among the FIs, and (4) compared the optimal thresholds to the U.S. Environmental Protection Agency's RWQC.

■ MATERIALS AND METHODS

Site Description. Water quality data obtained for the Chicago Health Environmental Exposure and Recreation Study (CHEERS) were used for this analysis. CHEERS was a prospective cohort study that found an association between limited-contact recreation on the Chicago Area Waterways System (CAWS) and on general use waters (GUW) in and around Chicago. The CAWS is a heavily engineered waterway that receives secondarily treated, though nondisinfected, effluent from three wastewater treatment plants.[39,40] Because of wastewater discharge, only limited-contact recreational activities (e.g., boating, canoeing, and kayaking) are permitted in most of the CAWS. CHEERS found that the incidence of gastrointestinal illness among limited contact recreators was approximately 14/1000 greater than the risk among nonwater recreators (cyclists, joggers, etc.).[41]

Sample Collection. Samples were collected during the water recreation seasons of 2007−2009. Samples for measurement of FIs were taken from surface waters by direct grab sampling. Samples for measurement of protozoan pathogens were taken from surface waters by large volume (20 L) continuous flow centrifugation according to EPA Method 1623.[23] Captured particles were washed once with 5 mL of elution buffer (5-fold concentrated phosphate-buffered saline, 0.05% Tween 80, and 100 μL of Antifoam A) and resuspended in 10 mL of elution buffer to yield a final sample, concentrated 2000-fold.[23,24] All FI samples were collected within 1 h of pathogen samples, at the same location and on the same day, with the exception of two observations, which were collected within 2 h.

Sampling locations were classified as (1) CAWS, (2) GUW, or (3) other. CAWS locations included the Cal-Sag Channel; the North Branch, South Branch, and Main Stem of the Chicago River; and the North Shore Channel.[42] GUW locations included three beaches on Lake Michigan (Jackson Park, Leone, and Montrose), three harbors on Lake Michigan (Belmont, Jackson Park, and Montrose), six small lakes (Busse, Crystal, Arlington, Maple, Mastodon, and Tampier), Lovelace Park Pond, the Skokie Lagoons, and multiple sites along the Des Plaines and Fox Rivers.[42] Samples taken from the North Branch Dam were designated as "other".

Microbiological Methods. Surface water samples were put on ice and transported to commercial laboratories for analysis.[43] Microbial analyses were EPA Method 1623 for *Giardia* and *Cryptosporidium*[23,43] and EPA Method 1602 for F+ and somatic coliphage[43,44] (all of which were performed at Scientific Methods, Inc., Granger, IN). *E. coli* and enterococci were analyzed by EPA Method 1603 and EPA Method 1600, respectively, at Microbac Laboratories (Merrillville, IN).[43,45,46] The concentration of enterococci was also determined using real-time qPCR as described in EPA Method A; this technique quantifies target DNA according to the comparative cycle threshold calculation method.[43,47] Samples that showed substantial inhibition on qPCR analysis (inhibition is defined as a difference between the cycle threshold sample processing control in unknown and calibrator samples of $\geq$3) were excluded from data analysis. Quality control measures included field blank samples, field split samples, and spiked samples to measure analytical recovery.[43] Culture-based measures of *E. coli* and enterococci are denoted with (cx), and qPCR-based measures are denoted with (q).

Detection limits were one (oo)cyst per 20 L for both *Giardia* and *Cryptosporidium*, 1 PFU per 100 mL for F+ coliphage, 10 PFU per 100 mL for somatic coliphage, and 1 CFU per 100 mL for both *E. coli* (cx) and enterococci (cx). For enterococci (q), the lowest reportable value was considered to be 1 CCE. Samples below the detection limit were assigned a value that was $^{1}/_{10}$ of the lowest detectable concentration: 0.1 CFU/100 mL for *E. coli* and enterococci, 1 PFU/100 mL for somatic coliphage, and 0.1 PFU/100 mL for F+ coliphage. A standard ROC method is a nonparametric rank-based method that imposes no distribution assumptions on the data set.[34] Thus, the use of any single substitution value (one-half or one-tenth the detection limit) would not affect our results because the rank of each value would not change.

Data Set Preparation. A total of 293 sets of time- and location-matched FI and pathogen samples were collected. Of

Table 1. Fecal Indicator and Protozoan Pathogen Detection Rates and Densities (N = 198)[a]

	fecal indicators					pathogens	
	enterococci (cx)	*E. coli* (cx)	somatic coliphage	F+ coliphage	enterococci (q)	*Giardia*	*Cryptosporidium*
percent detected	100%	100%	76%	64%	99%	74%	36%
units	CFU/100 mL	CFU/100 mL	PFU/100 mL	PFU/100 mL	CCE/100 mL	no./20L	no./20L
mean	510	1700	670	19	10000	31	2.1
geometric mean	130	320	39	1.4	2200	1.7	0.10
range	0.81−18000	0.55−23000	1.0−37000	0.10−480	0.10−130000	0.025−360	0.025−70

[a]Samples below the detection limit were assigned a value that was $^1/_{10}$ of the lowest detectable concentration for these statistics.

these, 105 sets were collected once per day at a location (e.g., unique location dates), while on 95 location days, sets of samples were collected twice per day (approximately 6 h apart). Within-day measures of a microbe were correlated with one another ($\rho \geq 0.7$ for the FIs, $\rho = 0.9$ for *Giardia*, and $\rho = 0.5$ for *Cryptosporidium*), suggesting that within-day samples cannot be treated as independent observations (Table 1 of the Supporting Information). In a standard ROC analysis, observations that are dependent can, in extreme cases, lead to class imbalance and bias the diagnostic value of a classifier.[48,49] We obviate the problem by averaging microbe densities that occur on the same day and in the same location to yield a single input into the ROC curve. For the purposes of this analysis, protozoan pathogen samples were identified as being either present or absent. When the data for *Giardia* and *Cryptosporidium* were averaged, a measurement was recorded as absent only if both observations that day were below the detection limit. The within-day averaging decreased the number of time- and location-matched FI−pathogen samples from 293 to 198. Each of the 198 observations contained the sampling date and location; densities of *E. coli* (cx), enterococci (cx), somatic coliphage, F+ coliphage, and enterococci (q); and descriptors of the presence of *Giardia* and *Cryptosporidium*.

Statistical Analysis. Descriptive statistics were calculated using SAS version 9.2 (SAS Institute Inc., Cary, NC). Normality was tested using the D'Agostino-Pearson test, and log normality was tested by applying the same test to log-transformed microbe densities. All correlations were calculated using Spearman's correlation (ρ) (Table 2 of the Supporting Information).

Receiver-Operating Characteristic Analysis. MedCalc software was used for ROC analysis (MedCalc version 12.3.0.0 for Windows, MedCalc Software, Mariakerke, Belgium). Briefly, to implement the ROC analysis, FI densities were ranked and each density was used, in turn, as a threshold. At each threshold, the numbers of true positives (TPs), false positives (FPs), true negatives (TNs), and false negatives (FNs) were tabulated, and the sensitivity [TP/(TP + FN) × 100%] and specificity [TN/(TN + FP) × 100%] were calculated. A TP is an event in which the FI density is greater than the threshold and the pathogen is present. An FP is an event in which the FI density is greater than the threshold and the pathogen is absent. A TN is an event in which the FI density is lower than the threshold and the pathogen is absent. An FN is an event in which the FI density is lower than the threshold and the pathogen is present.

ROC curves were created by plotting sensitivity and 100 − specificity as a pair of (x, y) coordinates on a Cartesian plane. The optimal threshold is the FI density that maximizes the sensitivity and specificity concurrently. Graphically, the optimal threshold corresponds to the point on the ROC curve geometrically closest to perfect classification, 100% specificity and 100% sensitivity [(x, y) = (0, 100)]. Quantitatively, this point can also be determined using the Youden Index, or the maximal average of sensitivity and specificity.[50] Once the point closest to perfect classification was identified, the sensitivity and specificity, and corresponding FI density, were established.

ROC curves were calculated for the five FIs with respect to each of the protozoan pathogens, for a total of 10 ROC curves. FI densities at the optimal thresholds were compared to the 2012 RWQC.[9] We also calculated the positive predictive value [+PV = TP/(TP + FP)] and negative predictive value [−PV = TN/(TN + FN)] of the optimized FI density thresholds.

The area under the ROC curve (AUC) is interpreted as the probability that a given test (FI density) correctly diagnoses a TP condition with respect to a TN condition.[51] In this study, the AUC was calculated for each curve and the standard error was derived according to the method presented by Delong et al.[52] Binomial exact confidence intervals were calculated for each AUC. We tested the null hypothesis that a diagnostic test was no better than chance alone at correctly predicting the presence or absence of a given pathogen in surface water (for H_0, AUC = 0.5) at $\alpha = 0.05$. The null hypothesis value (AUC = 0.5) corresponds to the event that the ROC curve is the line $y = x$. In other words, at each FI density threshold, the numbers of TP and FP are equal and the FI density has no predictive power. Differences between AUC values determined for the five FIs paired with the same pathogen were also evaluated using the method of Delong et al.[52] considering the correlations arising from ROC curves derived from the same cases. The Delong method uses the theory on generalized U statistics to generate an estimated covariance matrix to evaluate statistical differences of areas under correlated ROC curves. This approach is nonparametric and imposes no distribution assumptions on the data set.[52]

■ RESULTS

Descriptive statistics for the five FIs and two pathogens are listed in Table 1. The D'Agostino−Pearson test rejected normality for all microbe measures, with the exception of log-transformed measurements of enterococci (cx) and *E. coli* (cx). *Giardia* was detected more frequently (74%) than *Cryptosporidium* (36%). Concordance between the presence and absence of *Giardia* and *Cryptosporidium* occurred in 61% of samples. Of the 198 observations, 113 (57%) were from CAWS locations, 61 (31%) from GUW, and 24 (12%) from other (Table 3 of the Supporting Information). All geometric mean density and detection frequency of all microbes at GUW locations were less than or equal to those at CAWS and other locations.

Results of the ROC curves are depicted in panels a and b of Figure 1, and the AUCs are listed in Table 2. All ROC curves yielded AUC values statistically significantly greater than 0.5, indicating that the FIs performed better than chance alone at

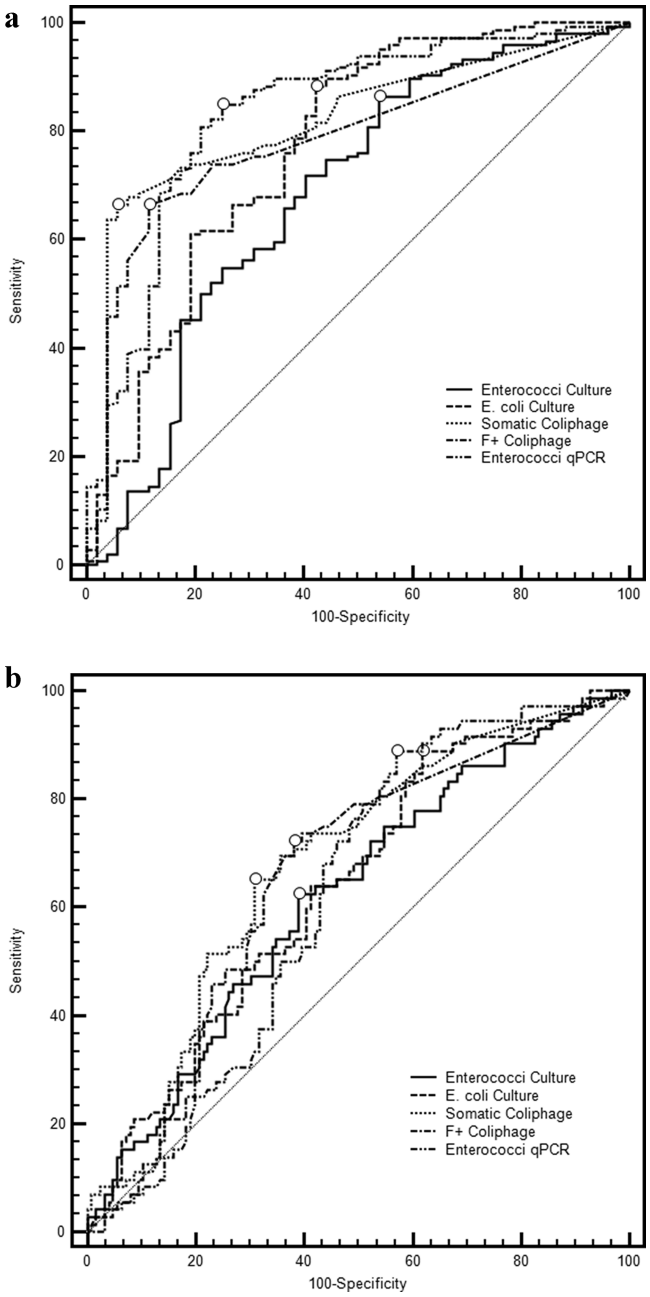

Figure 1. (a) ROC curves constructed for *Giardia* and enterococci (cx), *E. coli* (cx), somatic coliphage, F+ coliphage, and enterococci (q). The line Y = X indicates a lack of association (AUC = 0.5), and the empty circle indicates the point corresponding to the optimal threshold. (b) ROC curves constructed for *Cryptosporidium* and enterococci (cx), *E. coli* (cx), somatic coliphage, F+ coliphage, and enterococci (q).

predicting the presence and absence of protozoan pathogens. For *Giardia*, enterococci (cx) was the least informative classifier (AUC of 0.68; 95% CI from 0.61 to 0.75), while enterococci (q) was the best (AUC of 0.84; 95% CI from 0.78 to 0.89). For *Cryptosporidium*, the test for somatic coliphage was the most informative classifier (AUC of 0.68; 95% CI from 0.61 to 0.74). Qualitatively, the indicators were better predictors of the presence and absence of *Giardia* than of the presence and absence of *Cryptosporidium*; that is, the AUC of an indicator test of the presence and absence of *Giardia* was always higher than when the same tested diagnosed the presence and absence of *Cryptosporidium*.

The optimal thresholds identified in each of the 10 ROC curves are listed in Table 3. The magnitude of sensitivity and specificity vary among the FI−pathogen tests. For all FI−*Giardia* pairs, the +PV at the threshold was greater than the −PV. This demonstrates that in the current setting, the prediction of the presence of *Giardia* was better than the prediction of the absence of *Giardia*. Given prevalence rates of >50% for *Giardia*, beach management decisions that deem waters unsafe for primary contact recreation are more likely to be accurate than decisions that allow swimming. The opposite was true for *Cryptosporidium*, which has a prevalence rate of <50%. With respect to that pathogen, the −PV at the optimal threshold was greater than the +PV. The ROC analysis interpolated the optimal thresholds of F+ coliphage for *Giardia* and *Cryptosporidium* to be 0.6 and 0.8 PFU/100 mL, respectively. Both thresholds fall between the F+ coliphage detection limit (1 PFU/100 mL) and the substitution for a nondetect (0.1 PFU/1 mL). In such a case, the optimal threshold should be the measurement closest to optimal cutoff (0, 1).

The area under the curve (AUC) is a quantitative measure of the ability of the indicator microbe to differentiate TN (pathogen absence) and TP (pathogen presence) conditions. In our analysis, data for indicators were derived from the same set of water samples. The ROC curves generated from tests performed on the same cases are inherently correlated.[52] This correlation means that the confidence intervals of the AUCs cannot be compared to determine statistically significant differences. Instead, the method of Delong et al.[52] was used to test the statistical significance of differences between AUCs for the 10 possible pairs of indicators with respect to *Giardia* and *Cryptosporidium* (Tables 4 and 5 of the Supporting Information, respectively). Enterococci (cx) (AUC = 0.68) proved to be a statistically inferior test of the presence of *Giardia* compared to the tests for *E. coli* (cx) (AUC = 0.77), somatic coliphage (AUC = 0.82), F+ coliphage (AUC = 0.79), and enterococci (q) (AUC = 0.84). Enterococci (q) was statistically superior to the enterococci (cx) and statistically superior to *E. coli* (cx). No two indicator tests showed statistically significant diagnostic differences for *Cryptosporidium*.

Culture-based measurement of FIs in recreational waters has been widely criticized because of the >24 h time lag between sample collection and the result. Other methods like qPCR are more rapid and allow for same-day management decisions. The ROC analysis shows that enterococci measured by qPCR were at least as sensitive as enterococci measured by culture for predicting the presence of both protozoan pathogens, and more specific at predicting the absence of *Giardia* (Table 3).

In summary, we found that all five FIs meaningfully predicted the presence and absence of protozoan pathogens in Chicago area surface waters used for recreation, indicated by AUC values of >0.5 (Table 2). In all cases, FIs were better predictors of the presence and absence of *Giardia* than the presence and absence of *Cryptosporidium*, but not all FIs predicted the presence and absence of pathogens equally well. At the optimal thresholds (Table 3), enterococci (q), F+ coliphage, and somatic coliphage were relatively sensitive and specific for predicting the presence and absence of pathogens.

Table 2. Areas Under the ROC Curve (AUC) for Fecal Indicators with Respect to *Giardia* and *Cryptosporidium*

	Giardia				
	enterococci (cx)	*E. coli* (cx)	somatic coliphage	F+ coliphage	enterococci (q)
AUC (95% CI)	0.68 (0.61, 0.75)	0.77 (0.70, 0.83)	0.82 (0.76, 0.87)	0.79 (0.72, 0.84)	0.84 (0.78, 0.89)
standard error	0.047	0.042	0.032	0.033	0.035
	Cryptosporidium				
	enterococci (cx)	*E. coli* (cx)	somatic coliphage	F+ coliphage	enterococci (q)
AUC (95% CI)	0.62 (0.55, 0.69)	0.64 (0.57, 0.70)	0.68 (0.61, 0.74)	0.65 (0.58, 0.72)	0.62 (0.55, 0.69)
standard error	0.041	0.040	0.040	0.039	0.040

Table 3. Optimal Thresholds for Fecal Indicators with Respect to the Presence of Absence of *Giardia* and *Cryptosporidium* and Sensitivity, Specificity, +PV, and −PV at Each Threshold

	Giardia				
	optimal threshold	sensitivity (95% CI)	specificity (95% CI)	+PV (95% CI)	−PV (95% CI)
enterococci (cx) (CFU/100 mL)	32	86 (80, 91)	46 (32, 61)	82 (75, 88)	56 (39, 70)
E. coli (cx) (CFU/100 mL)	70	88 (82, 93)	58 (43, 71)	85 (79, 91)	64 (49, 77)
somatic coliphage (PFU/100 mL)	31	66 (58, 74)	94 (84, 99)	97 (92, 99)	50 (40, 60)
F+ coliphage (PFU/100 mL)	0.6	66 (58, 74)	89 (77, 96)	94 (88, 98)	48 (38, 59)
enterococci (q) (CEE/100 mL)	1200	85 (78, 90)	75 (61, 86)	90 (84, 95)	64 (51, 76)
	Cryptosporidium				
	optimal threshold	sensitivity (95% CI)	specificity (95% CI)	+PV (95% CI)	−PV (95% CI)
enterococci (cx) (CFU/100 mL)	170	63 (50, 74)	61 (52, 70)	48 (38, 58)	74 (65, 82)
E. coli (cx) (CFU/100 mL)	96	89 (79, 95)	38 (30, 47)	45 (37, 54)	86 (74, 94)
somatic coliphage (PFU/100 mL)	170	65 (53, 76)	69 (60, 77)	55 (44, 65)	78 (69, 85)
F+ coliphage (PFU/100 mL)	0.8	72 (60, 82)	62 (53, 70)	52 (42, 62)	80 (70, 87)
enterococci (q) (CEE/100 mL)	1200	89 (79, 95)	43 (34, 52)	47 (38, 56)	87 (76, 94)

Table 4. ROC Optimal Threshold Values Compared to the 2012 Recreational Water Quality Criteria (RWQC)

	ROC-derived thresholds		EPA-recommended threshold 1[a]		EPA-recommended threshold 2[b]	
	Giardia	*Cryptosporidium*	GM[c]	STV[d]	GM[c]	STV[d]
enterococci (cx) (CFU/100 mL)	32	170	35	130	30	110
E. coli (cx) (CFU/100 mL)	70	96	126	410	100	320
enterococci (q) (CCE/100 mL)	1200	1200	not available	1000[e]	not available	640[e]

[a]Recommendation 1: estimated illness rate (NGI) of 36 per 1000 primary contact swimmers. [b]Recommendation 2: estimated illness rate (NGI) of 32 per 1000 primary contact swimmers. [c]Geometric mean (GM). [d]Statistical threshold value (STV). [e]Recommended beach action value.

■ DISCUSSION

Indicator organisms in water are not solely derived from human fecal pollution, and the density of FIs and the diversity of species depend on hydrogeological and environmental conditions, as well as sources of fecal material.[28,53] Some important determinants include the temporal variability due to the effects of sunlight;[54] differential growth and survival of indicators in soil,[30] sand,[27,32] and sediment;[31] the presence of macro-alga[29] and seagull feces;[55] and hydrogeological variability.[28] Thus, it is probable that epidemiologic relationships between FI density and health risk are of limited generalizability. Under provisions of the 2012 RWQC, local water management agencies can develop scientifically defensible site-specific standards.[10]

Given this relatively new support for site-specific focus on recreational water quality by the EPA, the use of pathogen detection to optimize FI-based criteria is appealing. The presence of pathogens is a risk for public health because pathogens, including *Giardia* and *Cryptosporidium*, are tied to outbreaks or may indicate the presence of other pathogens. While pathogen sampling and analytical methods remain time-consuming and may be prohibitive for inclusion in an epidemiologic study,[9,10] pathogen sampling may be feasible at specific locations and provide beach managers another metric of recreational water quality that is related to health.

FI thresholds identified in this study were generally comparable to the 2012 U.S. EPA RWQC (Table 4), which are based on prospective cohort epidemiologic studies.[9,10] Though the pathogens to which water recreators in the EPA studies were exposed are unknown, the comparison affirms that the ROC analysis can result in plausible inference about water quality. Additional studies in other settings are required to evaluate whether the reasonable concordance between ROC-derived FI thresholds and epidemiologically derived FI criteria observed herein can be generalized. In addition, further research is required to establish the density of pathogens or their presence and absence for quantitative microbial risk assessment of recreational waters.

ROC analysis is a flexible technique. While we have applied ROC analysis to predict the presence and absence of protozoan pathogens, ROC analysis can be used to find FI thresholds that predict values that exceed or comply with other risk-based thresholds, applied to other pathogens, or used with other

predictors of water quality, such as rainfall.[38] In addition, different weights can be assigned to sensitivity and specificity, to reflect policy preferences. In our analysis, we assigned equal importance to sensitivity and specificity, but public health protection could be enhanced by weighting sensitivity (detection of pathogens) over specificity (avoiding swim bans when pathogens are absent).

Other statistical methods can be and have been used to determine FI thresholds for water quality. For example, tree regression was used by Wilkes et al.[16,56] to predict *Cryptosporidium* in watersheds based on environmental and land use variables. Logistic regression is another obvious candidate. While it is possible to determine the sensitivity and specificity of fitted tree and logistic regression models, neither approach considers sensitivity and specificity explicitly in model fitting. Tree regression models typically split the data at thresholds to maximize the difference between the two groups, while logistic regression may minimize the differences between observed and predicted values. It is possible to implement trivial tree and logistic regression models to predict the presence and absence of pathogens from a single variable, FI density, but the real power of these approaches comes from their ability to explicate complex systems described by diverse variables. In contrast, ROC analysis requires no information other than FI density and data about the presence and absence of pathogens.

■ ASSOCIATED CONTENT

Supporting Information

Spearman (ρ) rank correlation coefficients between same-day-location measures of microbes (Table 1), Spearman (ρ) rank correlation coefficients between all microbe measures for the final data set (Table 2); location group geometric means (Table 3), and differences between AUC values for *Giardia* (Table 4) and *Cryptosporidium* (Table 5) between each possible pair of FI tests. 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.

■ ABBREVIATIONS

AUC	area under the curve
CAWS	Chicago Area Waterway System
CCE	calibrator cell equivalents
CHEERS	Chicago Health Environmental Exposure and Recreation Study
CI	confidence interval
CFU	colony-forming units
U.S. EPA	U.S. Environmental Protection Agency
FI	fecal indicator
FN	false negative
FP	false positive
GM	geometric mean
GUW	general use waters
PFU	plaque-forming units
qPCR	quantitative polymerase chain reaction
ROC	receiver-operating characteristics
STV	statistical threshold value
TN	true negative
TP	true positive
UIC	University of Illinois at Chicago

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