

Enterococci as Indicators of Lake Michigan Recreational Water Quality: Comparison of Two Methodologies and Their Impacts on Public Health Regulatory Events

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The frequency of poor-water-quality advisories issued in Milwaukee and Racine, Wisconsin, in the absence of identifiable sources of contamination brought into question the reliability of the present indicator organism, *Escherichia coli*. Enterococci have been suggested as an alternative to *E. coli* for freshwater monitoring due to their direct correlation to swimmer-associated gastroenteritis. The purpose of this research was threefold: (i) to explore enterococci as an alternative to *E. coli* for monitoring freshwater Lake Michigan beaches, (ii) to evaluate the impact of the two indicators on regulatory decisions, and (iii) to compare membrane filtration m-enterococcus agar with indoxyl- β -D-glucoside to a chemical substrate technique (Enterolert) for the recovery of enterococci. Recreational water samples from Milwaukee ($n = 305$) and Racine ($n = 153$) were analyzed for the enumeration of *E. coli* and enterococci using IDEXX Colilert-18 and Enterolert. Correlation between the indicators was low ($R^2 = 0.60$ and 0.69). Based on U.S. Environmental Protection Agency bacterial indicator threshold levels of risk for full body immersion, using enterococci would have resulted in 56 additional unsafe-recreational-water-quality advisories compared to the total from using *E. coli* and the substrate-based methods. A comparison of the two enterococcal methods ($n = 124$) yielded similar results ($R^2 = 0.62$). This was further confounded by the frequent inability to verify enterococci from those wells producing fluorescence by the defined substrate test using conventional microbiological methods. These results suggest that further research is necessary regarding the use of defined substrate technology interchangeably with the U.S. Environmental Protection Agency-approved membrane filtration test for the detection of enterococci from fresh surface water.

In 1999, the U.S. Environmental Protection Agency (USEPA) set forth an Action Plan for Beaches and Recreational Waters, as Americans faced the risk of illness associated with swimming in surface waters contaminated with disease-causing microorganisms (20). Previous epidemiological studies performed by the USEPA demonstrated a direct relationship between the density of *Escherichia coli* and enterococci in surface waters and an increase in swimmer-associated gastroenteritis (18). Limits were established as guidelines for recreational water quality based on this information. For freshwater, the present single-sample advisory limits are 235 CFU/100 ml for *E. coli* and 61 CFU/100 ml for enterococci. The 5-day geometric mean should not exceed 126 CFU/100 ml for *E. coli* and 33 CFU/100 ml for enterococci (16). During 1997 to 1998, a total of seven states, including Wisconsin, reported eight outbreaks of waterborne disease, specifically gastroenteritis, associated with recreational water that affected over 1,000 individuals (4). Reliable indicator tests are needed to predict known etiological agents that have been isolated from surface waters and proven to cause outbreaks of gastrointestinal illness (*E. coli* O157:H7, Norwalk-like virus, and *Cryptosporidium parvum*) and to predict events in which the agent was never identified (4).

The cities of Racine and Milwaukee, Wisconsin, 30 miles apart, have a total of five regulated, freshwater Lake Michigan beaches, i.e., North and Zoo beaches in Racine and Bradford, McKinley, and South Shore beaches in Milwaukee. While the state of Wisconsin has a model beach ordinance based on USEPA guidelines for fecal coliform bacteria levels, there is no mandate regarding the frequency of testing and local jurisdictions presently are not required to monitor bathing beaches (23a). However, Milwaukee and Racine have been testing Lake Michigan recreational water quality for more than 20 years, using fecal coliforms and recently an *E. coli* standard (from 1999) in accordance with published USEPA guidelines (18). Although the frequency of testing increased from twice weekly to daily (Monday through Friday) in 1999, there has been no appreciable change in the number of closures or advisories posted in Racine or Milwaukee (Table 1).

Historically, fecal coliforms and *E. coli* have been used as indicators of choice when monitoring recreational water quality (6). Recent studies have shown that high densities of *E. coli* and enterococci recovered from recreational waters have a stronger correlation with swimming-associated gastrointestinal disease than do densities of fecal coliform bacteria (3). Although enterococci have been traditionally used to monitor marine bathing water (12), both of these indicators have been referenced as being equally acceptable for monitoring freshwater (1, 6). Therefore, studies of both marine water and

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TABLE 1. Annual recreational-water-quality closures and/or advisories posted since 1994 in Racine and Milwaukee^a

Yr	Days/% for:		No. of days in Racine beach season	Days/% for South Shore Beach	Estimated no. of days in Milwaukee beach season
	North Beach	Zoo Beach			
1994	5/6	21/25	84	18/20	90
1995	51/59	42/48	87	12/13	90
1996	5/5	2/2	95	10/11	90
1997	18/19	30/32	93	15/17	90
1998	16/16	4/4	98	21/23	90
1999	15/16	19/20	94	32/36	90
2000	62/66	39/41	94	42/47	90
2001	17/20	21/25	84	No data	90
2002	27/31	40/46	87	No data	90

^a Bradford and McKinley beach data are not shown. The fecal coliform standard was used until 1998. The *E. coli* standard has been used since 1999. Milwaukee posts advisories based on bacterial density and environmental factors. Racine went to an advisory system (good/poor) in 2001 in order to deter patrons from swimming in nonlifeguarded areas when the designated beach area was closed due to high bacterial counts. Days/% = number of days closed or posted/% of total beach season closed (estimated for Milwaukee).

freshwater have been undertaken to support the idea that enterococci may be the more relevant indicator of water quality (6). The ideal characteristics of a bacterial water quality indicator are as follows: their density in water can be positively correlated with potential health risks associated with exposure, they should demonstrate similar resistance to environmental stress as pathogens, and they are detectable by simple and inexpensive assays.

To investigate the use of enterococci as an indicator of recreational water quality, the city health department laboratories of Racine and Milwaukee tested routine recreational surface water samples for the presence of enterococci against their standard indicator, *E. coli*. Both indicator organisms were compared using the defined substrate based detection methods from IDEXX Laboratories, Westbrook, Me., i.e., the methylumbelliferyl- β -glucuronide (MUG)-based Colilert-18 for *E. coli* and Enterolert for enterococci.

Additionally, two methods for the 24-hour detection of enterococci in fresh surface waters were compared: a recently described variation of the conventional membrane filtration method (11) and a commercially available defined substrate method (Enterolert; IDEXX Laboratories, Inc.). In summary, the purpose of this research was to compare *E. coli* against enterococci as indicators of pollution at five designated swimming beaches in southeast Wisconsin and to compare two presently available methods for the detection of enterococci in fresh surface waters.

MATERIALS AND METHODS

Sample collection. Freshwater samples were collected 5 days per week (Monday through Friday) from each of the five designated swimming beaches (North, Zoo, Bradford, McKinley, and South Shore) in sterile screw-top bottles or sterile Whirl-Pak bags (Nasco). Samples were obtained in the morning by wading out to an approximate 1-m depth (high deep) and taking a 200- to 300-ml sample from 0.3 m below the surface of the water. Samples were transported in a cooler on ice packs to the respective laboratories. Split samples were tested within 1 h of collection for the presence of enterococci and/or *E. coli*.

Enumeration of *E. coli* by Colilert-18. Both Milwaukee and Racine Health Department Laboratories used IDEXX Colilert-18, a chemical detection method, for the recovery of *E. coli* according to previously established laboratory protocols. Undiluted freshwater samples or a 1:10 dilution made with sterile deionized water was each mixed with reagent and placed in a Quantitray/2000 according to the manufacturer's instructions (Colilert-18 product insert; IDEXX

Laboratories). Quantitrays were sealed and placed in a 35°C incubator for 18 h. A quality control organism (*E. coli* ATCC 25922) was run once daily to validate test performance. Following incubation, Quantitray wells were read for yellowness revealing *o*-nitrophenyl- β -D-galactopyranoside hydrolysis and fluorescence, which indicate MUG cleavage, with the aid of a UV light box (366 nm). The number of wells producing fluorescence was compared to the manufacturer-provided most-probable-number (MPN) table to enumerate *E. coli* in terms of MPN/100 ml.

Verification of *E. coli* isolates from Colilert-18 and Quantitray/2000 wells. Verification of *E. coli* was performed by cleaning the back of a Quantitray well with a 70% alcohol pad and withdrawing its contents with a sterile syringe and needle. Several drops of the withdrawn material were plated to MacConkey agar and *E. coli* broth with MUG. Following 24 h of incubation at 35°C, the MacConkey plates were examined for lactose utilization. After 24 h of incubation at 44.5°C in a water bath, the *E. coli* broth with MUG was examined for gas production and MUG activity. Previous studies performed by the Milwaukee and Racine Health Department Laboratories have shown that Colilert-18 is an acceptable substitute for the traditional membrane filtration test using m-total *E. coli* (m-TEC) agar (J. Kinzelman, A. Singh, C. Ng, R. Bagley, and S. Gradus, Abstr. Great Lakes Beach Conf., p. 5, USEPA—Region 5, 2001).

Enumeration of enterococci by membrane filtration. Undiluted samples or a 1:10 dilution of freshwater samples was filtered through 0.45- μ m-pore-size graded membrane filters (Millipore) onto m-enterococcus agar with indoxyl- β -D-glucoside (mEI agar) prepared according to USEPA specifications (19, 21). The addition of indoxyl- β -D-glucoside, nalidixic acid, 0.1 N NaOH, and triphenyltetrazolium chloride to mEI agar (Difco) (13) allowed for a single 24-h incubation period at 41°C. Quality control was run daily to validate test performance and included both positive (*Enterococcus faecalis* ATCC 33012 and *E. faecium* ATCC 35667) and negative (*E. coli* ATCC 25922 and *Serratia marcescens* ATCC 43862) controls. Colonies producing a blue halo were counted under $\times 10$ magnification with the aid of a Quebec colony counter.

Enumeration of enterococci by Enterolert. Undiluted freshwater samples or a 1:10 dilution made with sterile deionized water was each mixed with reagent and placed in a Quantitray/2000 according to the manufacturer's instructions (Enterolert product insert; IDEXX Laboratories). Quantitrays were sealed and placed in a 41°C incubator for 24 h. Quality control was run daily to validate test performance and included both positive (*E. faecalis* ATCC 33012 and *E. faecium* ATCC 35667) and negative (*E. coli* ATCC 25922 and *S. marcescens* ATCC 43862) controls. The number of wells producing fluorescence under UV light at 366 nm was compared to the manufacturer-provided MPN table to enumerate enterococci in terms of MPN/100 ml. It should be noted that the decision to dilute samples was determined by the previous day's bacterial level and that a 10-fold dilution was not routinely performed with Enterolert, as is the manufacturer's recommendation for marine waters. There is no manufacturer's recommendation regarding dilutions for analyzing freshwater, and the use of one would have often resulted in values below the detection limit of this test system.

Verification of enterococcal isolates from Enterolert wells. Verification of enterococci was performed by subculturing to standard microbiological media for the isolation of gram-positive organisms and rapid biochemical tests. The back of a Quantitray well was disinfected with a 70% alcohol pad, and the contents were withdrawn with a sterile syringe and needle. Several drops of the withdrawn material were used to inoculate sheep's blood agar (BAP), colistin-nalidixic acid agar (CNA), and brain heart infusion broth (BHI). After 24 h of incubation at 35°C, colonies on BAP and/or CNA were tested for characteristic reactions with the following rapid biochemical tests: catalase (–), Lancefield group D (+), and pyrrolidonyl arylamidase (+). BHI tubes exhibiting growth were subcultured to bile esculin agar for an additional 24 h and were examined for characteristic black colonies. Selected colonies from the 24-hour old BAP were transferred to identification panels (Remel, Inc., Lenexa, Kans.) in an attempt to identify the organism to the genus and/or species.

Consideration should be given when comparing results obtained by the chemical substrate method to the colony counts resulting from membrane filtration. The IDEXX Quantitray/2000 system incorporates 97 wells in a single tray with an upper limit of detection equaling 2,419.2 MPN/100 ml. The manufacturer-stated 95% confidence limits for Quantitray/2000 are similar to those published for membrane filter counts (6; <http://www.idexx.com>), and theoretically the defined substrate method should be a satisfactory substitute for traditional membrane filtration.

Statistical analysis of these results were performed to answer two questions: (i) whether *E. coli* and enterococci could be used interchangeably as regulatory water quality indicators for freshwater and (ii) given the equal incubation times, whether Enterolert could be used interchangeably with membrane filtration on mEI agar as a detection technique.

A total of 458 Lake Michigan surface water samples from Milwaukee ($n =$

TABLE 2. Statistical analysis of raw data comparing enterococcus and *E. coli* values recovered from Lake Michigan, summer 2001^a

Location	<i>n</i>	<i>df</i>	Pearson's coefficient (<i>r</i>)	<i>r</i> ²	<i>P</i>
Milwaukee	305	303	0.83	0.69	<0.001
Racine	153	151	0.78	0.60	<0.001

^a The total number of samples from Racine reflect only those samples that also had paired enterococcal results on mEI agar.

305) and Racine (*n* = 153) were analyzed for the enumeration of *E. coli* and enterococci by using IDEXX products (Colilert-18 and Enterolert). The total number of samples collected per municipality varied due to the length of the sampling season and number of sampling days per week. Paired data were analyzed for correlation using the Texassoft WINKS 4.6 statistical software program (Table 2). Statistical analysis did not show a strong relationship between the concentrations of these two indicator organisms in the Lake Michigan surface water samples tested during the summer of 2001. Linear regression of log-converted data suggest that statistically *E. coli* and enterococci may not be used interchangeably as indicators of recreational water quality to receive similar regulatory results ($R^2 = 0.60$ at Racine; $R^2 = 0.69$ at Milwaukee).

Over a period of approximately 2 months, 124 split samples of surface water from Racine were analyzed using IDEXX Enterolert and mEI agar to determine if these methods were comparable in their ability to recover enterococci from fresh surface water. Statistical analysis of log-converted data comparing these two methods was performed using Texassoft WINKS 4.6. A *t* of 1.97 along with a *P* of ~0.06 indicates that the means of these two methodologies are not equal and in fact are significantly different. Linear regression of log-converted data demonstrated relatively poor dispersion about the trend line ($R^2 = 0.62$) (graph not shown). This was most obvious when there was no bacterial growth on the mEI agar plate but when the IDEXX Enterolert detected enterococci and in instances when the Enterolert reached its maximum level of detection (2,419 MPN/100 ml).

RESULTS

Enterococci versus *E. coli* as indicators of recreational water quality. Statistical analysis suggests that *E. coli* and enterococci were not always comparable as indicators of recreational water quality. The regulatory implications, i.e., beach water quality advisories, of enterococci versus *E. coli* were evaluated based on USEPA recommendations for (i) single-sample allowable-maximum-density guidelines of 235 CFU per 100 ml for *E. coli* and 61 CFU per 100 ml for enterococci (6) and (ii) the geometric mean of no less than five 1-day samples within a 30-day period equal to 126 CFU per 100 ml for *E. coli* and 33 CFU per 100 ml for enterococci (10) (Table 3).

According to the single-event guidelines, in Milwaukee and Racine, the enterococcal threshold was exceeded 20 and 46

more times, respectively, than the *E. coli* threshold. This would have resulted in 13 additional unsafe-water-advisory days in Racine. Data just for Racine are shown in Table 4. (Note: in actual practice, Racine uses both the single-event and 5-day geometric mean criteria for issuing advisories while Milwaukee uses single-event indicator levels as well as rainfall events to determine advisories.)

Based on the 5-day geometric mean standards alone, 33 MPN/100 ml for enterococci and 126 MPN/100 ml for *E. coli* (22), Racine and Milwaukee would have considered their beaches unsafe for swimming 33 and 26 additional times, respectively.

Enterolert versus mEI agar as a detection method for enterococci in freshwater. Several limitations were observed for both the Enterolert and membrane methods. For the membrane filtration method, interference may occur due to overgrowth by background bacteria or false negatives may occur due to the presence of substances (e.g., high turbidity) that may clog the pores of the filter. Also, it has been noted that false-positive results can occur using the membrane filtration technique (1). Although defined substrate technology may be a potentially more sensitive test that is less prone to interference due to overgrowth by background bacteria or false negatives due to the presence of substances, in our study, among wells producing fluorescence by the Enterolert method, approximately 50% of the aseptically removed material produced satisfactory organism identifications when subjected to conventional biochemical testing (data not shown). Unfortunately, there were also several occurrences when material removed from the Quantitray wells failed to grow bacteria when plated to a conventional medium selective for gram-positive organisms (e.g., CNA or BAP) or a combination of BHI broth and blue esculin agar. This phenomenon was seen more frequently in Quantitray wells exhibiting a lesser degree of fluorescence. Therefore, it may have contributed to the disparity in the percentage of confirmations. Ability to verify and identify enterococcal isolates to the species level may have given us an indication whether or not they were of a species more prevalent in polluted waters (1) or were autochthonous in the environment.

DISCUSSION

The evolution of beach testing methodologies and detection of indicators of human pathogens and indirectly human illness

TABLE 3. Number of samples collected per site, range of values for both indicators, number of incidents where indicator levels exceeded the 5-day geometric mean (GM), and number of incidents of single-sample limit being exceeded according to USEPA guidelines for enterococci and *E. coli* and defined substrate technology (IDEXX Colilert-18 and Enterolert)

Site or total	<i>n</i>	<i>E. coli</i> minimum no.	<i>E. coli</i> maximum no.	Enterococcus minimum no.	Enterococcus maximum no.	No. of incidents where levels exceeded the 5-day GM for:		No. of incidents where levels exceeded the single-sample limit for:	
						<i>E. coli</i> (>126 CFU/100 ml)	Enterococci (>33 CFU/100 ml)	Enterococci (>61 CFU/100 ml)	<i>E. coli</i> (>235 CFU/100 ml)
Bradford Beach	102	<1	2,419.2	<1	2,419.2	22	39	29	20
McKinley Beach	102	<1	2,419.2	<1	2,419.2	4	14	9	8
South Shore Beach	101	<1	2,419.2	<1	2,419.2	82	92	62	52
North Beach	211	<1	24,192	<1	5,475	0	21	47	21
Zoo Beach	152	<1	10,462	<1	2,420	6	22	50	30
Total	666					114	188	187	131

TABLE 4. Levels of the recreational-water-quality indicators *E. coli* and enterococci isolated by defined substrate (Colilert-18 and Enterolert) and membrane filtration and solid agar (m-TEC and mEI agars) for the months of July and August 2001 at Racine, Wisconsin

Date	Results for different North Beach locations in July and August 2001													
	North Beach 1			North Beach 2			North Beach 3			North Beach 4				
	<i>E. coli</i> (m-TEC, CFU/100 ml)	Enterococci (mEI, CFU/100 ml)	<i>E. coli</i> (Colilert-18, MPN/100 ml)	Enterococci (Enterolert, MPN/100 ml)	<i>E. coli</i> (Colilert-18, MPN/100 ml)	Enterococci (Enterolert, MPN/100 ml)	<i>E. coli</i> (m-TEC, CFU/100 ml)	Enterococci (mEI, CFU/100 ml)	<i>E. coli</i> (Colilert-18, MPN/100 ml)	Enterococci (Enterolert, MPN/100 ml)	<i>E. coli</i> (m-TEC, CFU/100 ml)	Enterococci (mEI, CFU/100 ml)	<i>E. coli</i> (Colilert-18, MPN/100 ml)	Enterococci (Enterolert, MPN/100 ml)
July														
Thursday, 12	<10	<10	10	4	31	3	<10	<10	<10	<10	<10	<10	31	3
Monday, 16	<10	<10	<10	1	<10	1	<10	<10	<10	<10	10	<10	<10	2
Tuesday, 17	90	20	169	32	211	66	2,20	1,840	5,475	2,419	1,370	1,230	2,613	2,419
Wednesday, 18	310	100	554	236	480	2,419	210	70	489	144	270	130	733	534
Thursday, 19	<10	<10	10	1	31	6	<10	<10	20	5	<10	<10	20	20
Monday, 23	<10	10	<10	8	31	31	<10	150	337	153	30	10	<10	20
Tuesday, 24	<10	20	10	6	20	8	<10	<10	52	7	30	10	98	33
Wednesday, 25	570	820	620	2,419	2,046	2,419	1,300	850	1,201	2,419	820	1,140	703	1,300
Thursday, 26	210	50	318	318	474	474	230	210	691	691	370	80	638	638
Monday, 30	20	<4	10	2	20	1	4	12	52	2	12	20	20	1
Tuesday, 31	4	4	<10	3	10	5	24	4	41	2	4	20	<10	7
August														
Wednesday, 1	4	4	10	1	10	1	4	4	10	2	28	8	52	8
Thursday, 2	10	<10	20	1	20	3	20	90	122	18	50	20	72	11
Monday, 6	<10	10	10	2	<10	1	<10	<10	<10	1	<10	<10	<10	6
Tuesday, 7	20	10	10	1	40	1	10	90	120	20	10	<10	74	4
Wednesday, 8	10	20	41	4	52	1	30	<10	74	4	30	60	72	28

have not kept pace with public health demands to rapidly, accurately, and efficiently predict human health risk factors. Previous research performed jointly by Milwaukee and Racine concluded that Colilert-18 was an acceptable method for the isolation of *E. coli* from surface freshwater samples when compared to the traditional membrane filtration method using m-TEC agar (Kinzelman et al., Abstr. Great Lakes Beach Conf.). Limited references existed comparing the recovery of enterococci from a solid agar such as mEI versus chemical substrate methodologies (5, 8, 10, 23) (Enterolert—a rapid method for the detection of *Enterococcus* spp., publication no. 12E, IDEXX Laboratories, Inc.). We have attempted to evaluate a method infrequently used to monitor freshwater, i.e., substrate utilization for enterococci, a bacterial indicator of fecal contamination, by comparing two methods for the recovery of enterococci from surface waters. Those two methods are conventional membrane filtration and substrate utilization; they were compared to determine what, if any, advantages exist with the defined substrate method.

There were drawbacks in using Enterolert besides the lack of correlation between methodologies. Most important of these was the inability to reculture and verify isolates as enterococci, implying the possibility of false-positive reactions in some of the wells. However, there are also advantages to using this system, which included decreased incubation time (from 48 to 24 h), ease of use, and minimal technical training required of personnel. When mEI agar becomes commercially available, the two methods will be equal with respect to incubation times. Previous research mainly focuses on the use of this product in a marine environment. We feel that further research into recovery and verification of isolates would be preferable before drawing any final conclusions regarding the use of these methods interchangeably for freshwater testing. It should be noted that Enterolert is awaiting USEPA approval as a method for ambient surface water testing (9).

In this study we attempted to evaluate what regulatory impact an alternative indicator would have on the number of water quality advisories issued per beach season. With respect to the use of *E. coli* or enterococci as indicators of recreational freshwater quality, the statistical data did not support the hypothesis that either indicator could be used interchangeably in recreational freshwater monitoring. The use of enterococci would have resulted in more unsafe-water advisory postings. These additional unsafe-water advisories may or may not be indicative of increased protection of public health since enterococci may be of fecal or plant origin (15, 24), including filamentous algae that are autochthonous in the environment. Racine and especially Milwaukee experience drifts of *Cladophora* spp., a type of filamentous algae, washing up on the foreshore sands (2, 17). Growth can occur in this nutrient-rich environment and could potentially pollute foreshore sands and surface water (R. Whitman, unpublished data).

Although enterococci are used routinely and successfully in monitoring marine waters, only six states, one tribe, and one territory use enterococci as a standard for recreational freshwater monitoring (22). Additionally, due to the limited sample size, we did not calculate our own one-sided confidence limit (75 to 95%) based on beach usage but rather used the published value of 61 CFU/100 ml as the single-event advisory limit. This could affect the number of advisory events incurred

using enterococci instead of *E. coli*. Of the states using enterococci as an indicator of recreational water quality, both freshwater and marine water, several deviate from this standard (16). At this point, we believe that the use of enterococci would not provide additional protection to the public with regard to exposure (7, 14) and that additional unsafe-water advisories would have a negative impact on public perception and the local economy. Further studies elucidating the relationship of enterococci to human illness and pathogens in recreational water would be helpful.

The state of Wisconsin, while not mandating the frequency of testing or choice of indicator organism at this time, does have a model beach code whose recommendations parallel USEPA guidelines for recreational water testing. The implementation of a monitoring program and the decision to close a beach or post an unsafe-water-quality advisory are left to the discretion of local authorities. Presently, in those communities that do routinely test, either fecal coliform bacteria or *E. coli* is used as indicators of surface water quality. Fecal coliform bacteria were the indicator organisms of choice for routine monitoring in both Milwaukee and Racine in the past. Due to *E. coli*'s direct correlation to swimmer-associated gastroenteritis and the fact that other fecal organisms such as *Klebsiella* spp. can be found in the environment irrespective of recent fecal contamination, both communities have switched to an *E. coli* standard.

The sources of pollution to recreational waters are many and varied with causes often challenging investigators. While point sources may be easily identified, such as the combined sewer overflows in Milwaukee or storm sewer outfalls in Racine, they are often not easily rectified. Nonpoint sources, such as the widespread presence of waterfowl on public beaches, remain even more elusive. The number of advisories may fluctuate dramatically from year to year with no predictability or correlation to specific events such as rainfall. Therefore, it is the recommendation of the authors that all communities adjacent to bodies of freshwater used for recreational purposes adopt some form of recreational water-testing program. Further research of Lake Michigan surface waters, possibly using mEI agar or other USEPA-approved methods, would be necessary before public health laboratories in either Racine or Milwaukee would use enterococci as an indicator of freshwater quality. Based on the results of this study, we recommend 5-day-a-week testing with *E. coli* as the preferred indicator according to USEPA guidelines, until such time as definitive research shows that an alternative indicator is more protective of the public health.

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